ANIMALS

Research across a broad range of animal systems areas is urgently needed for the future enhancement of animal production efficiency, as well as to address such areas as the modification of animal products. To accomplish this, grants are awarded in four broad areas of research: (1) animal reproductive biology, (2) cellular growth, development and nutrient utilization of animals, (3) animal molecular genetics and gene mapping, and (4) animal health and well-being. Emphasis is given to innovative approaches to research questions related to animals primarily raised for food or fiber. This includes aquaculture species and those animals such as horses that contribute significantly to the agricultural enterprise of the country.

ANIMAL REPRODUCTIVE EFFICIENCY

Panel Manager - Dr. Charlotte Farin, North Carolina State University
Program Director - Dr. Debora L. Hamernik

The primary objective of this program area is to increase our knowledge of reproductive biology in agriculturally important animals with the goal of increasing reproductive efficiency. This program supports innovative research on: (1) factors controlling ovarian function including follicular development, ovulation, and corpus luteum formation and function, (2) factors controlling male reproduction, (3) gamete physiology, including oogenesis and spermatogenesis, gamete maturation, and mechanisms regulating gamete survival *in vivo* or *in vitro*, (4) mechanisms affecting embryo development and survival, mechanisms of embryo-maternal interactions, embryo-implantation, development of optimal culture methods for embryos, and causes and remediation of early embryonic loss, (5) mechanisms involved in placental function, and (6) parturition, postpartum interval to conception, and neonatal survival.

Because alterations in animal behavior and animal well-being may impair fecundity, this program also encourages research on the mechanisms controlling animal responses to physical and biological stresses that impinge upon reproductive processes. Research should contribute to an understanding of the causes, consequences, and avoidance of stress, rather than merely describing the physiological effects of stress on reproductive efficiency.

9802420 Cellular and Molecular Organizational Events Affecting Porcine Uterine Capacity
Bartol, F. F.
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Grant 98-35203-6198; $162,000; 3 Years

Factors affecting embryo mortality dictate litter size in pigs. Normal embryos are lost when uterine capacity to support conceptus development is limited. Uterine capacity depends upon the ability of the uterine endometrium to respond to conceptus and maternal signals. This may be determined, in part, by the success of events supporting uterine organization during early postnatal life. The postnatal uterine organizational program can be disrupted by administration of estradiol valerate (EV) to pigs between birth and postnatal day (PND) 56. Normal and disrupted endometrial development are estrogen receptor (ER)-dependent, and age-specific postnatal effects of EV reflect changes in spatial patterns of endometrial ER expression. Reduced embryo survival in adults exposed to EV from PND 0-13 indicates that disruption of EV-sensitive, ER-dependent postnatal uterine development may affect subsequent uterine function. Research will determine effects of EV-induced disruption of uterine development during specific neonatal periods, defined by the spatial distribution of endometrial ER, on subsequent cellular and molecular uterine responses to: [1] estrogen and progesterone in prepubertal gilts; and [2] natural endocrine and local conceptus signals in adult gilts during complementary phases of the estrous cycle and early pregnancy. Studies constitute the first tests of the functional importance of estrogen-sensitive neonatal uterine organizational events, and will establish the utility of a new experimental strategy for identification of developmental determinants of uterine capacity in swine. Research supports USDA goals to improve reproductive efficiency in livestock by understanding and identifying factors affecting embryo survival.

9801948 Comparative Study on Limits to Efficient Estrogen Synthesis in Pigs and Cattle
Conley, A. J.
University of California, Davis; Department of Population Health and Reproduction; Davis, CA 95616
Grant 98-35203-6439; $190,000; 3 Years

Estrogens, the hormonal steroids formed from androgens in the pre-ovulatory ovarian follicle, are involved in coordinating virtually all aspects of reproduction. These steroids are necessary for fertility and many conditions limiting reproductive efficiency, including silent estrus, delayed ovulation, cystic ovarian disease, ovulation failure and follicular atresia are accompanied by an imbalance of androgen and estrogen synthesis. This project will examine key enzyme complexes responsible for synthesizing these hormones in cattle and pigs which exhibit essential species differences in these catalytic processes. Expression of recombinant bovine and porcine enzymes using virus and bacterial expression systems will provide both standards for quantification of tissue levels and active proteins for *in vitro* function studies. Ovarian follicular activities and levels of protein components constituting each enzyme complex, will be determined in tissues and compared to those of complexes formed from recombinant protein components reconstituted *in vitro*. Proposed experiments will determine which step limits efficient estrogen production in the pre-ovulatory follicle, and what protein components of enzyme complexes, including essential and accessory redox partners, influence the efficiency of hormone synthesis. These analyses will evaluate both anatomical compartments of the follicle and identify similarities and/or differences between these species. Thus, the anatomical site and biochemical limits to estrogen synthesis will be determined in cattle and pigs, focusing future research on strategies to optimize ovarian steroidogenesis and minimize infertility caused by conditions associated with steroid hormone imbalances.

9802421 Vth International Symposium on Reproduction in Domestic Ruminants

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Colorado State University; Animal Reproduction and Biotechnology Laboratory; Ft. Collins, CO 80523-1682
Grant 98-35203-6023; $9,000; 3 Months

The symposium, planned by a committee representing ten countries, will bring together the latest information on reproductive physiology and reproductive management of domesticated ruminants, including cattle, sheep, goats, deer, water buffalo, and camelids. The symposium will serve as a forum for comparing how reproductive functions are similar or different among these economically important species. Specific sessions include: 1)Follicular Development, 2)Neuroendocrine Relationships, 3)Comparative Reproductive Function: Implications for Management, 4)The Corpus Luteum, 5)Male Function and Fertility, 6)Embryonic Survival, 7)Local Cellular and Tissue Communication, 8)Nutrition and Metabolic Signaling, and 9)Reproductive Technology. In addition, two poster sessions will be held. The symposium will also provide an opportunity to compare techniques, experimental designs, analytical methods, environmental and ethical concerns, and ways to maximize utilizations of research resources. It will provide an opportunity for exchange of the latest information among the leading scientists in countries and research groups around the world. Proceedings will be published as a supplement to the *Journal of Reproduction and Fertility*. Thus, critical, state-of-the-art information will rapidly become available to thousands of scientists in the United States and throughout the world. New collaborations will be forged among attendees, thus broadening comparative approaches and increasing the rate of progress of our understanding of the reproductive processes and the application of that understanding to reproductive management of domestic ruminants. The progress is extremely critical to the successful and economical production of animal protein to feed the continually increasing human population.

9801978 Molecular Control of Luteal Secretion of Progesterone in Ruminants

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Colorado State University; Animal Reproduction and Biotechnology Laboratory; Ft. Collins, CO 80523-1682
Grant 98-35203-6376; $175,000; 2 Years

The long-term goal of the proposed research is to reduce the nearly 30% embryonic wastage which occurs as a result of inadequate secretion of progesterone in domestic ruminants. Recently a molecule was identified (steroidogenic acute regulatory protein [StAR]) which appears to play a key role in the rate-limiting step of progesterone biosynthesis, which is the transport of cholesterol from the outer to the inner mitochondrial membrane. This is also the step where acute stimulatory and inhibitory regulation of this process appears to occur. Therefore, the proposed research focuses on regulation of the gene encoding StAR. Specific Aim 1 addresses regulation of the StAR gene by positive and negative stimuli. The promoter region of the gene will be isolated and sequences conferring basal and regulatory promoter activity determined in small and large steroidogenic luteal cells. Research for Specific Aim 2 focuses on translational regulatory mechanisms including synthesis and phosphorylation of StAR. Phosphorylation of StAR is associated with modified capabilities to transport cholesterol and rates of progesterone synthesis. Ewes will be injected with luteinizing hormone (LH) or prostaglandin F2alpha (PGF_{2a}) during the mid-luteal phase of the estrous cycle and the total quantity and phosphorylation state of StAR determined. LH and PGF_{2a} are the primary positive and negative hormonal regulators of luteal function, respectively. The effects of luteotropic and luteolytic stimuli on the quantity and phosphorylation state of StAR will be determined.

The proposed research addresses (a) embryo survival and embryo maternal interactions, and (b) control of ovarian function, corpus luteum formation and function. After a complete understanding of the factors which regulate progesterone secretion is obtained, research to utilize this information to reduce embryonic wastage due to inadequate progesterone can be initiated.

9802166 Oxytocin and Molecular Mediators of Luteolysis in Sheep

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Strengthening Award; Grant 98-35203-6635; $170,000; 2 Years

The corpus luteum is formed from the ovulatory follicle in the ovary and secretes progesterone, the hormone which maintains pregnancy in mammals. Knowledge of the function of the corpus luteum is critical for understanding the reproductive cycle and for improving fertility in domestic ruminants which are important food and fiber species in US agriculture. In sheep, the corpus luteum, in addition to producing progesterone, also produces large amounts of oxytocin, a peptide hormone originally identified in the posterior pituitary gland. Oxytocin secreted by the corpus luteum is thought to amplify uterine pulses of prostaglandin F2 alpha ($PGF_{2\alpha}$) which cause regression of the corpus luteum (luteolysis). Experiments are designed to deplete oxytocin from the corpus luteum to determine if luteal oxytocin is essential for normal regression. The precise effects of depleting luteal oxytocin will be determined by (1) the rate of decline of progesterone during luteolysis; (2) the length of the ovarian cycle; (3) the magnitude of uterine PGF_{2a} pulses and (4) the rate of follicular development, which may provide an index of fertility in the following cycle. Collaborative studies will also be carried out with investigators at the Universities of Connecticut and New Hampshire to determine the molecular mechanisms by which PGF_{2a} blocks the synthesis of progesterone in the corpus luteum and how PGF_{2a} causes structural regression of the corpus luteum at the end of the reproductive cycle. Because the corpus luteum plays a pivotal role in controlling the reproductive cycle and in the establishment of early pregnancy, such studies have important practical implications for increasing reproductive efficiency in domestic animals.

9801965 Targeting of Polyunsaturated Fatty Acids in Antiluteolytic Diets to Improve Embryo Survival

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Grant 98-35203-6367; $200,000; 3 Years

A major cause of poor reproductive efficiency in dairy cattle is sub-optimal embryo survival. Specific long chain polyunsaturated fatty acids, including eicosapentaenoic acid (EPA) and docasahexaenoic acid (DHA) in fish oils, can decrease prostaglandin F2 alpha (PGF_{2a}) synthesis in mammalian tissues. Goals of this project are to determine the mechanism by which fish meal decreases PGF_{2a} secretion and to test whether feeding fish meal increases pregnancy rates of lactating dairy cows. Our hypothesis is that feeding fish meal will increase embryo survival by reducing uterine secretion of PGF_{2a} and contribute to maintenance of the corpus luteum for pregnancy. The first objective is to determine the level of fish meal in the diet that decreases estradiol and oxytocin-induced secretion of PGF_{2a}. The second objective is to determine how fish meal inhibits synthesis of PGF_{2a} in the endometrial component of the uterus. The ability of various stimulants of PGF_{2a} to increase secretion of PGF_{2a} from *in vitro* cultures of endometrial explants from cows fed fish meal or a control diet will be measured. These *in vitro* responses will be coupled with measurements of gene expression of enzymes involved in the synthesis of PGF_{2a}. Uterine tissue concentrations of EPA and DHA will be related to actual activity of the tissues to produce PGF_{2a}. Our third objective is to determine if the optimal dietary level of fish meal will enhance pregnancy rates in lactating dairy cows. The project exemplifies technology transfer to enhance competitive and sustainable food production from cattle and is readily applicable to the cattle industry.

9801984 More Efficient Production of Transgenic Salmonids by Germ Line Manipulation
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Strengthening Award; Grant 98-35203-6225; $175,000; 3 Years

Farmed raised rainbow trout is a substantial and growing animal industry in the United States. Genetic improvements in growth rate, feed efficiency and disease resistance are likely through the use of modern molecular biology with the resultant development of transgenic lines of trout. In the production of a transgenic fish, the gene of interest is injected into the fertilized egg; normally this newly introduced gene is incorporated into only one chromosome. In order to have true breeding broodstock, this founder animal is crossed through two generations. In all, this process of transgenic broodstock development in trout takes a minimum of six years. The objective of this investigation is to develop methodologies to substantially reduce the time required for this process. One mechanism is to produce female founder animals; allow them to spawn, fertilize their eggs with spermatozoa that have no functional DNA, and double the chromosome number derived exclusively from the egg. The second mechanism is to double the number of chromosomes following the incorporation of the transgene in male embryos. At sexual maturity the male founder animals are expected to produce sperm with two chromosome sets; this sperm will be used to fertilize eggs with no functional DNA to produce the true breeding broodstock. The first of these methods is easier to accomplish but limited to females; the second is faster because sexual maturity can be accelerated in male trout. In both cases the goal is to incorporate new developments in molecular genetics into functional broodstock in a shorter period of time.

9801126 Contemporary and Emerging Issues in Animal Physiology
Hurley, W. L.
American Dairy Science Association; Savoy, IL 61874
Grant 98-35203-6026; $5,000; 1 Year

The project is in partial support of the physiology program to be held at the Joint American Dairy Science Association and American Society of Animal Science Meetings in July, 1998, in Denver, Colorado. The conference program will enhance the overall research efforts of U.S. agriculture by providing a forum for synergistic interaction among animal physiologists
and other animal scientists with diverse research interests from all parts of the world. The program encompasses many aspects of animal reproductive physiology, mammary gland biology and milk synthesis, and other areas of animal physiology. The program is organized to focus on contemporary and emerging areas of research in animal physiology. In addition, program planning is aimed at maximizing discussion and participation by the audience, choosing internationally recognized speakers, and using non-traditional approaches in program organization and presentation. The core of the conference is a series of mini-symposia, each with two to three internationally recognized speakers who will provide state-of-the-art talks. Planned sessions include mini-symposia on "Emerging Reproductive Technologies," "Male Reproduction," "Metabolic Regulation of Reproduction," and "Cell Turnover in Reproductive Processes". A roundtable discussion on "Plasticity of Mammary Gland Function" will also be held. The overall program includes poster sessions presenting current research by the international animal physiology communities.

9802359 Nuclear and Cytoplasmic Maturation of Prepubertal Bovine Oocytes
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Grant 98-35203-6275; $188,000; 3 Years

The increase in production of milk by the nation's dairy herds has been due primarily to the increased genetic potential of the cows brought about by the introduction of artificial insemination and use of frozen semen. These techniques allowed the widespread use of

sires of exceptional genetic merit in all herds. The continued success of this program is dependent on our ability to identify cows that can be used as the dams of future sires. While techniques have been developed to accurately evaluate the genetic potential of cattle, the factor limiting annual genetic gain is the length of the generation interval, that is, the age at which progeny are born. With the development of assisted reproductive technologies in cattle, such as *in vitro* maturation, fertilization and culture of eggs and embryos, it might be possible to collect eggs from prepubertal heifers and fertilize them *in vitro* and subsequently transfer the embryos to recipient cows. Preliminary data indicates that large numbers of eggs can be collected from prepubertal heifers, but few eggs develop in culture. The objectives of this work are to identify why these eggs lack developmental competence. Techniques such as micromanipulation of oocytes (injection of compounds known to induce maturation) and determining the activity of key enzymes in the maturation and fertilization of eggs will provide basic information that could be incorporated into new methods for *in vitro* maturation, fertilization and culture of prepubertal bovine oocytes. This would allow calves to be used as egg donors and could shorten the generation interval in cattle by up to 50%. It is important that productive efficiency per cow continues to increase as the nation's dairy herd continues to decrease at a time when population and the total demand for dairy products is increasing.

9801979 Neural Mechanisms Mediating Progesterone-Induced Suppression of LH Release in Sheep
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Postdoctoral Fellowship; Grant 98-35203-6309; $85,000; 2 Years

To optimize reproductive efficiency in domestic livestock it is crucial to understand the mechanisms governing release of luteinizing hormone-releasing hormone (LHRH). This hormone is produced and secreted from the brain and acts on the pituitary gland to induce release of LH. The steroid hormone progesterone plays the key role in regulating the pattern of LHRH, and thus LH secretion during a specific phase of the estrous cycle. The pulsatile pattern of LHRH release during this phase is essential for proper development of ovarian follicles prior to ovulation. Despite its prominent role in regulating LHRH, the neural mechanism(s) through which progesterone acts is unknown. The specific objectives of this work are to determine whether progesterone 1) alters activity of all or a subpopulation of LHRH neurons, 2) alters activity of neurotransmitter systems known to influence LHRH neurons, and 3) acts directly on these neuronal systems to influence their activity. Using the sheep as the model, these possibilities will be addressed with techniques that allow for assessment of progesterone-induced changes in synthesis of LHRH and neurotransmitters of interest and localization of receptors for progesterone within these neuronal systems. The results will provide new information concerning the neural pathway through which progesterone regulates LHRH release and may ultimately provide a means for increasing reproductive efficiency in domestic livestock.

9801943 Mechanisms of Zinc and Calcium Regulation of Sperm Storage in the Turkey
King, L. M.
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Postdoctoral Fellowship; Grant 98-35203-6221; $85,000; 2 Years

Turkey hens can successfully store sperm in specialized sperm storage tubules (SST) for up to 70 days, whereas sperm can only be maintained for 6-8 hr at 4C without significant declines in fertility. Since commercial turkey production relies exclusively on artificial insemination, improving sperm storage would have a significant impact on this industry. The mechanism of sperm storage within the SST is unknown but is thought to include reversible suppression of sperm respiration and motility. Therefore, factors important in the regulation of sperm motility may be involved in long-term sperm storage in the turkey hen. The metal ions zinc and calcium have been shown to affect sperm motility in several species, and may be involved in sperm storage in the hen. These studies will investigate how zinc and calcium affect sperm motility in the turkey. Additional studies will examine how the sperm storage tubules (SST) contribute to long-term survival of sperm by using isolated SST in co-culture with sperm. The final studies will use zinc and calcium in the sperm-SST co-cultures to determine how these ions affect survival of sperm in the storage tubules of the turkey. By understanding the basis of sperm storage in the hen, it might be possible to develop similar storage conditions in the laboratory. Adaptation of this knowledge by the turkey industry could dramatically improve artificial insemination practices by increasing sperm survival, and by reducing the number of sperm needed for insemination. This would translate into savings of millions of dollars annually in reduced animal, labor and management costs, with consequent savings to the consumer.

9802006 Role of Matrix Metalloproteinases in Ovulation
Smith, G. W.; Pursley R.
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New Investigator Award; Grant 98-35203-6226; $155,000; 2 Years

Reproductive efficiency is a major factor limiting the economic success of livestock operations. Rupture of a mature ovarian follicle and subsequent release (ovulation) of a viable egg is a crucial determinant of fertility. The preovulatory luteinizing hormone (LH) surge initiates the ovulatory process. Degradation of the extracellular matrix of the follicle wall is necessary for follicle rupture and egg release to occur. However, the intrafollicular mechanisms that control ovulation are not completely understood. The matrix metalloproteinases (MMPs) are a large family of enzymes that degrade specific components of the extracellular matrix. Administration of MMP inhibitors after the LH surge inhibits ovulation. Thus, the MMPs likely help mediate the ovulatory process. Our overall objective is to elucidate

the mechanisms whereby the LH surge causes ovulation in dairy cattle. Our working hypothesis is that the LH surge increases synthesis and subsequent activation of the MMPs. To test our hypothesis, we will investigate the effect of the LH surge on localization, activation and activity of selected MMP family members (interstitial collagenase, gelatinase A, gelatinase B, stromelysin 1 and the newly discovered membrane type 1 metalloproteinase) within bovine preovulatory follicles. Elucidation of the mechanisms that regulate expression and activity of MMPs during the periovulatory period will greatly enhance our understanding of the intrafollicular regulation of ovulation. This information may ultimately lead to development of improved methods to control ovulation and hence increase reproductive efficiency in dairy cattle.

9801940 Matrix Metalloproteinases and their Inhibitors in Ovine Corpora Lutea

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Grant 98-35203-6282; $150,000; 2 Years

Following ovulation in sheep, a postovulatory follicle (~30 mg) develops into a corpus luteum (~600 mg) within a few days. Accompanying this tremendous luteal tissue growth are dramatic changes in tissue remodeling including cell growth, proliferation, and migration. The preceding changes are accompanied by remodeling of the extracellular matrix (ECM) which consists of several proteins. Specific protein components of the ECM are cleaved by the matrix metalloproteinase (MMPs) family of enzymes and the activity of these enzymes is inhibited by tissue inhibitors of metalloproteinases (TIMPs). TIMP-1 is a multifunctional molecule that in addition to inhibiting MMP activity, reportedly stimulates cellular proliferation and stimulates progesterone production by several cell types including luteinizing granulosal cells. The overall hypothesis is that MMPs and TIMPs have an important role in developing and preserving a microenvironment conducive to luteal tissue formation and adequate progesterone secretion. Our long term objective is to determine the physiological role of MMPs and TIMPs in the regulation of corpus luteum function. The Specific Aims of this proposal are: 1) To determine which MMPs are present and to quantify MMP activities during luteal development, maintenance, and regression and, 2) Determine the effect of MMP-3 (Stromelysin) or rTIMP-1 on secretion of progesterone by *in vitro* perifused ovine luteal tissue. Regulation of ECM turnover is a precisely controlled process that maintains cellular organization and is likely important for luteal tissue formation, function, and regression.

9801998 Control of the Porcine Corpus Luteum by the IGF-System

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Grant 98-35203-6283; $190,000; 3 Years

This proposal describes experiments designed to examine further the physiological role of the insulin-like growth factor (IGF) system in controlling the corpus luteum (CL) in the pig. The results obtained from these studies should facilitate the process of development of new approaches to controlling the estrous cycle and overall fertility in female swine, thus improving reproductive efficiency in this economically important species. In the first specific aim, we propose to examine which cell-types within the CL express the mRNAs and synthesize the proteins for each of the IGF-system components (IGF-I, IGF-receptor and IGF-binding proteins) using histochemical techniques and light microscopy. This information is critical to our understanding of the cellular interactions involved in IGF-system actions in this tissue. In the second specific aim, we propose to examine the role of the IGF-receptor at different stages of the estrous cycle using both cell culture (*in vitro*) and whole animal (*in vivo*) approaches. Data obtained from this study should enable us to determine whether or not the IGF-receptor, which is present at all stages of the estrous cycle, is fully capable of transducing the IGF-I "signal" equally at all of these stages. Finally in the third specific aim, we propose to challenge porcine CLs with anti-IGF-I antibodies, IGF-binding proteins and IGF-I antagonists (*in vitro* and *in vivo*). These studies should provide a direct test of the physiological importance of endogenous (i.e. CL derived) IGF-I in controlling the porcine CL.

9802168 Role of Angiogenic Factors in Ovarian Function

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Grant 98-35203-6222; $170,000; 3 Years

Production of animals for meat is a multi-billion dollar industry in the U.S. alone, and reproductive efficiency has a major impact on the profitability of animal agriculture. The long- term goal of this continuing project is to determine the role of vascular growth (angiogenesis) in reproductive (ovarian) function. It appears that vascular endothelial growth factor (VEGF) is a major regulator of vascular growth in the developing corpus luteum (CL). A novel and exciting observation made during the current project period is that perivascular cells in the CL are the major cell types expressing VEGF. These perivascular cells continue to express VEGF throughout the luteal lifespan. Thus, we have recently suggested that VEGF-expressing perivascular cells are the primary regulators of luteal vascularization and also may be critical for maintenance of luteal vascular beds in mature and regressing CL. In addition, numerous studies provide strong support for the suggestion that LH stimulates the expression of VEGF by luteal tissues. We have hypothesized that LH regulates expression of VEGF by luteal perivascular cells, which is the focus of the present proposal. The proposed experiments will be conducted to evaluate effects of LH on VEGF expression by preovulatory follicles and CL. Specifically, the proposed experiments will examine the *in vivo* effects of LH on follicular and luteal VEGF expression during the follicular to luteal transition (Exp. 1) as well

as in growing and mature CL (Exp. 2). We also will evaluate the *in vitro* effects of LH on VEGF expression by follicular (Exp. 3) and luteal (Exp. 4) tissues and cell types.

9801985 Urea, Bovine Uterine Secretions, and Embryo Development

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Grant 98-35203-6274; $180,000; 3 Years

Dairy cows are typically fed high protein diets to support high milk production and consequently, blood urea concentrations are elevated due to normal protein metabolism. Unfortunately, pregnancy rates in cows are reduced when urea is high, but the mechanisms underlying the associated impairment of fertility are unknown. Previous work found that high blood urea levels were associated with alteration in uterine luminal pH and secretion. *In vitro* cultures of bovine uterine cells were established that not only maintained a marked pH gradient, but also responded to urea by altering pH and ion fluxes. Surprisingly, exposure of the cultures to urea resulted in enhanced output of prostaglandins (PG) that are detrimental to embryo development. A hypothesis to explain these observations is that urea interferes with the actions of a key enzyme, carbonic anhydrase (CA) inside the uterus and, thereby, affects uterine luminal pH with associated enhanced secretion of PG. Experiments *in vivo* in lactating cows will determine the direct effects of intravenous urea infusion on these parameters: pH of the uterus; secretion of proteins and PG in the uterus; and development of transferred embryos. An *in vitro* bovine endometrial culture system will examine: the interactions of urea, steroids and CA on uterine secretions (ions, PG, proteins) and effects of secretions on early embryos. Cumulatively, these experiments will enhance understanding of possible interference by urea with uterine secretions that, in turn, may impact embryo survival and reduce fertility. Identifying the mechanisms involved provides a basis for dietary strategies or other therapeutic measures to increase fertility. Improved reproductive performance is necessary for efficient milk production and to sustain the long-term viability and competitiveness of the dairy industry.

9802161 Peroxidative Fragmentation of Sperm Membrane Lipids During Maturation and Capacitation

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Grant 98-35203-6273; $111,130; 2 Years

The structural and functional integrity of the sperm plasma membrane is essential for normal fertilization in mammals. However, the special complement of molecular species, especially lipids, assembled into the sperm membrane during spermatogenesis, epididymal maturation and capacitation is a two-edged sword. While pre-disposing the sperm to membrane fusion events required for egg penetration, sperm lipids are also extremely labile to a variety of intrinsic and extrinsic factors. Because sperm membrane lipids are polyunsaturated, they are especially susceptible to free radical attack and peroxidative degradation. When carried to the extreme, this results in breakage or fragmentation of lipids and loss of membrane integrity. Enzymes present in semen and the female reproductive tract regulate fragmented lipids. Impaired regulatory control of fragmented membrane lipids may contribute to reduced male fertility by disrupting sperm function, while controlled accumulation of fragmented lipids during the acquisition of fertility (epididymal maturation and capacitation) may actually predispose the sperm plasma membrane to requisite membrane fusion events during fertilization. The purpose of this project is to characterize the production of peroxidatively fragmented lipids in mammalian sperm and to determine how their accumulation affects sperm function and fertility. Results have major implications for improved preservation of semen from dairy cattle and other domestic animals for use in artificial insemination, *in vitro* fertilization, and other assisted reproductive technologies. Developing procedures to control or eliminate fragmented lipids may help to maintain sperm viability *in vitro* and provide a rational, mechanistic basis for improving *in vitro* fertilization and related procedures.

9802003 Role of the Fas Antigen in Bovine Follicle Development and Atresia

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Grant 98-35203-6220; $175,000; 3 Years

In mammals, 99% of ovarian follicles that begin to develop undergo degeneration or atresia. Our long term goal is to determine the mechanism for the initiation and progression of ovarian follicular atresia in cattle. The cow is an economically important species in which a basic understanding of follicle development and atresia are key factors in our ability to control reproductive efficiency. The cow provides an ideal model for studies of follicle atresia because the dynamics of the phenomenon during the estrous cycle have been well characterized by studying the growth and regression of follicles *in vivo* by transrectal ultrasonography. It is possible to identify and obtain follicles at known stages of health and atresia for biochemical and molecular analyses. Granulosa and theca cells within the follicle can be readily separated and the characteristics of cells within individual follicles can be studied. We will investigate the potential role of the cell surface receptor, the Fas antigen, and its ligand (Fas ligand) in initiation of ovarian follicle atresia in cattle. Fas ligand induces cells to undergo a form of programmed cell death called apoptosis. We will investigate whether the level of expression of Fas antigen and Fas ligand in granulosa and theca cells and their responsiveness to Fas ligand-mediated apoptosis changes during follicle development and atresia. We will also study the effects of cytokines, interferon gamma and tumor necrosis factor, on the Fas pathway. Knowledge of factors regulating follicle atresia may suggest strategies to increase ovulation rate in cattle.

9802165 Anatomical Basis of Seasonal Plasticity in the GnRH System of the Ewe

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Grant 98-35203-6321; $204,000; 3 Years

Reproductive function in vertebrates is under direct control by the brain, specifically by a group of neurons that secrete the hormone gonadotropin-releasing hormone (GnRH). In seasonal breeders, such as sheep, changes in daylength act as 'nature's contraceptive' to limit reproduction to certain times of the year by altering GnRH output. Neural pathways represent the conduits through which environmental cues ultimately reach the GnRH neuron. Current evidence suggests that GnRH neurons receive a variety of different neural inputs and that the total number of inputs changes during the year - more inputs are present during the breeding season than during the non-breeding season. We believe that this change in input to GnRH neurons, a process of remodeling, represents a fundamental property of the seasonal brain. This proposal seeks to uncover the identity of the seasonally-changing inputs to GnRH neurons in the ewe by using a combination of highly sensitive microscopic and anatomical techniques. Using the same techniques we will also examine if glial cells, which are intimately associated with GnRH neurons, also exhibit changes at the same time that changes in synaptic input occur. Glial cells may play an important role in brain remodeling by acting to prevent certain neural inputs to GnRH neurons from occurring. Together, these studies will produce a comprehensive description of the types of neuroanatomical changes taking place within the GnRH system of the sheep. Identifying these changes can potentially lead to the development of more efficient methods of intervention to enhance reproductive activity.

9801952 Involvement of a Unique Inter-alpha-Trypsin Inhibitor in Establishment of Pregnancy

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Oklahoma State University; Department of Animal Science; Stillwater, OK 74078-6051
Grant 98-35203-6224; $183,000; 3 Years

Approximately 40% of the potential piglets are lost prior to parturition in the pig. Embryo survival in the pig depends upon continued support of progesterone secretion from corpora lutea present on the ovary and attachment of the placenta to the maternal uterine lining. Secretion of estrogen from the developing embryo is the signal to establish maintenance of functional corpora lutea throughout pregnancy and initiate attachment of the placenta to the uterine surface. Interactions between each embryo and the maternal uterus play a critical role in the potential litter size at term. Attachment of the pig placenta involves adhesion to the uterine microvillus glycocalyx, possibly through a glycoprotein expressed at the time of attachment. We have identified the glycoprotein as inter-alpha-trypsin inhibitor heavy chain 4 (IaIH). Expression of IαIH on the uterine epithelial surface may serve to bind the placenta and facilitate attachment throughout pregnancy. Involvement of the enzyme, kallikrein, in cleavage of IαIH may direct the time of placental attachment and also release kinin from kininogen to increase bloodflow to the uterus and maintain progesterone secretion from the corpora lutea. The objective of our study is to investigate the role of IaIH in attachment of the pig placenta and the possible function of kallikrein in regulating uterine function throughout pregnancy. Regulation of kallikrein secretion could play a critical role in establishment of pregnancy in the pig and embryonic survival to term. A better understanding of uterine factors involved with embryonic survival may provide methods to increase litter size in swine.

9801956 Conference on "Frontiers in Reproduction"

Schatten, G.
Oregon Regional Primate Research Center; Division of Reproductive Sciences; Beaverton, OR 97006
Grant 98-35203-6010; $10,000; 4 Months

During the past quarter century, advances in reproductive research using species of agricultural importance has been fantastic. The major goal of the course is to instruct promising PhDs, DVMs and MDs in the most sophisticated technologies useful for solving future problems in reproduction. Emerging questions regarding cloning and nuclear transfer, molecular regulation of embryonic gene expression, neuroendocrine control of reproduction, molecular basis of implantation, and reproductive immunology will be covered. This is an intensive six-week laboratory and lecture course for young, independent scientists and physicians and advanced postdoctoral fellows seeking comprehensive and sophisticated training in research strategies and state-of-the-art methods on cellular, immunological and molecular biological approaches for advancing research in reproduction. The course will consist of lectures from resident faculty and other invited speakers, discussions and informal seminars, laboratory exercises and demonstrations and one-on-one tutorials. The five lecture and laboratory modules are integrated to provide participants with broad exposure to the emerging problems in reproduction: Module 1--Gametogenesis and Fertilization {May 26-June 5}; Module 2--Early Development {June 6-June 10}; Module 3--Reproductive Immunology and Stem Cells {June 11-June 18}; Module 4--Signal Transduction and Gene Expression {June 19-June 23}; Module 5--Genetic Manipulations in Reproductive Endocrinology {June 28-July 4}.

9801997 Early Identification of Sex in White Sturgeon

Fitzpatrick, M. S.; Schreck, C. B.
Oregon State University; Department of Fisheries and Wildlife; Corvallis, OR 97331-3803
Grant 98-35203-6227; $94,000; 2 Years

Successful white sturgeon aquaculture relies on the separation of sexes to achieve different production goals. Males are produced for their meat and females are produced for their caviar. Therefore, it is essential to be able to identify the sex of sturgeon as early as possible such that production procedures can be optimized for either meat or caviar. Currently, white sturgeon growers identify the sex of individuals using surgery when the fish are 3 to 4 years old. Such surgical procedures carry a certain amount of risk of permanent injury to the fish, as well as a decrease in the efficiency of farm operations because the surgical procedure cannot be reliably carried out before the animals have been grown for several years. The proposed studies will determine if the surgical procedure can be effectively replaced by a less-invasive blood test, and whether this blood test, which measures the circulating levels of steroid hormones, can reliably identify the sex of individuals at an earlier age and under different culture conditions. One year old sturgeon from a California farm will be divided into two groups; one to be grown at Oregon State University and the other at the University of California at Davis. Blood samples will be taken from tagged individuals at 8 week intervals and assayed for hormones to determine if and when the sex can be identified. This work is significant because it will provide a simple tool that can be immediately put to use in sturgeon aquaculture. In addition, the technology developed in this project may have broad application in aquaculture and in recovery efforts for endangered wild sturgeon.

9801936 Capacitation and Acrosomal Satus of Equine Sperm During Cooled Storage and Cryopreservation

Meyers, S. A.
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New Investigator Award; Grant 98-35203-6584; $150,260; 2 Years

The overall goal of this project is to enhance the efficiency of equine artificial insemination by providing rationale for new methods of stallion semen management. Failure of capacitation, premature capacitation, increased acrosomal lability, or inappropriate acrosome reactions may impede sperm function and reduce stallion fertility which could result in the loss of millions of dollars to the breeding industry. The central hypothesis is that commonly used storage and cryopreservation methods used for stallion semen alter the efficiency of sperm to capacitate and subsequently undergo the acrosome reaction. The sperm protein hyaluronidase (PH-20) has been shown to play a role in sperm capacitation, and this new marker will be used extensively to monitor sperm functional competency. Experiments will demonstrate that stallion semen, whether fresh, cooled, or cryopreserved, undergoes functional changes associated with modification of baseline capacitation status. Capacitation will be monitored by changes in hyaluronidase activity, hyaluronidase cellular distribution, sperm protein tyrosine phosphorylation, and baseline and induced acrosomal exocytosis. Knowledge gained from the proposed studies will enhance our understanding of the biology of equine sperm capacitation and allow development of treatments that optimize semen storage and fertilization.

9800474 Mammalian Gametogenesis and Embryogenesis, Gordon Research Conference

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Grant 98-35203-5959; $5,000; 1 Year

An understanding of the fundamental principles underlying the development of gametes and preimplantation embryos is inextricably coupled to continued progress in agricultural efficiency, clinically assisted reproduction, promoting normal embryonic and fetal development, contraceptive development, and preservation of wildlife resources. Moreover, gametes and early embryos are excellent experimental models for basic studies on the molecular and cellular mechanisms of reproduction whose application extends to many areas of biomedical importance. The objective of the Gordon Conference on Mammalian Gametogenesis and Embryogenesis is to provide a fertile environment for exchanging information at the cutting edge of research in the fundamentals of mammalian gametogenesis and embryogenesis that will inspire the generation of new ideas and experimental approaches. This will accelerate the advancement of knowledge of fundamental principles and their practical application to improve the reproductive efficiency of domestic and agricultural animals. It is the long standing philosophy of Gordon Research Conferences to promote interactions among young and established investigators to invigorate progress in critical research areas such as mammalian gametogenesis and embryogenesis.

9801983 Interferon Tau-Induced Uterine Proteins Affect Peri-Implantation Conceptuses

Bazer, F. W.; Burghardt, R. C.
Texas A&M University; Center for Animal Biotechnology; College Station, TX 77843-2471
Grant 98-35203-6337; $230,000; 2 Years

In sheep and other ruminant species, interferon tau (IFNt) is the pregnancy recognition signal. This research will define mechanisms whereby IFNt influences uterine function, development of the embryo and its associated membranes and interactions between conceptus and uterus essential for establishment and maintenance of pregnancy. Osteopontin is a protein in uterine secretions that increases in response to IFNt. The proposed research will determine whether IFNt and/or progesterone control secretion of osteopontin by uterus and conceptus and osteopontin binding to its receptors (integrins) to: (1) stimulate development of the conceptus; (2) induce attachment of conceptus to uterine epithelium; and (3) regulate production of IFNt by the conceptus. Results from these studies are expected to increase our understanding of the roles of IFNt in conceptus development and survival, as well as signaling its presence to establish pregnancy.

Knowledge of these endocrinological and physiological mechanisms will be used in efforts to increase reproductive efficiency in sheep, goats and cattle, as well as other ruminant species having commercial value in animal agriculture.

9801989 Role of Endometrial Glands in Uterine Function

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New Investigator Award; Grant 98-35203-6322; $192,000; 3 Years

Infertility, pregnancy loss and intrauterine growth retardation are common problems that affect reproductive efficiency, health and development in livestock. A large percentage of these problems may be attributed to an inability of the uterus to support pregnancy. All mammalian uteri have endometrial glands which secrete a variety of substances. These secretions are hypothesized to support development of the conceptus (embryo and associated placental membranes) during pregnancy. The purpose of this research proposal is to define the role of endometrial glands in uterine function. In order to accomplish this purpose, we will use sheep that lack glands in the uterus (uterine gland knockout; UGKO). The objectives of the proposed studies are to use this UGKO sheep model to determine the role of endometrial glands in uterine function during the estrous cycle and pregnancy. The long-term goal of this research is to increase knowledge of uterine gland function in an effort to optimize the reproductive performance and efficiency of animal production agriculture. Knowledge gained from the proposed studies will be useful to design management, biotechnological and genetic applications aimed at enhancing production efficiency.

9802434 Studies of Maternal Recognition of Pregnancy

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Grant 98-35203-6223; $200,000; 2 Years

Reproductive efficiency currently limits livestock production; early embryonic loss is a major contributor to economic losses in production. Structural and functional analyses of selected uterine cells in early pregnancy are needed to identify cellular interactions and signaling events involved in embryo recognition and attachment in domestic farm species. An understanding of cellular interactions occurring within tissue-level compartments of the uterus and the signaling events between embryonic and maternal tissues is required to achieve the goal of increasing the number of robust offspring and pregnancies per breeding animal. Hormone-responsive uterine cells and embryonic vesicle culture systems that mimic their *in vivo* counterparts have been developed to identify these interactions. These systems provide novel opportunities to investigate the relationships between ovarian- and conceptus-driven modifications of uterine cells and signal transduction between conceptus and uterine cells
involved in embryo recognition and implantation. Specific objectives of the proposed research are to: 1) Establish the involvement of cell signaling between uterine epithelium and trophectoderm *in vivo* mediated by a class of molecules termed integrins which are involved in both adhesion and signaling and 2) Identify integrin-mediated signal transduction pathways involved in uterine epithelial cells and trophectoderm *in vitro*. State-of-the-art non-invasive vital imaging technologies will be employed to accomplish these objectives.

9801955 Sequences that Stabilize Estrogen Receptor mRNA in Ovine Endometrium and Their Applications

Ing, N. H.
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Grant 98-35203-6272; $210,000; 3 Years

To understand how the hormone estrogen carries out its actions, we will study its receptor, a cellular protein that binds estrogen. Our model is the inner lining of the sheep uterus, which is extremely sensitive to estrogen. To reduce endogenous levels of estrogen, sheep will be ovariectomized and injected with one low dose of estrogen. Previously, we demonstrated that estrogen receptor (ER) protein and its mRNA in the uterus were increased 3 to 5-fold in response to estrogen treatment. In addition, the activity of the ER gene was not increased but the stability of the ER mRNA was. Here, we will identify the sequences within the ER mRNA that are required for induction by estrogen. To accomplish this objective, we will transfect different segments of the ER mRNA attached to a non-regulated mRNA (glyceraldehyde phosphate dehydrogenase mRNA) into cells and see which of these synthetic RNAs is stable. We will also develop a model cell system, that up-regulates ER mRNA in response to estrogen (similar to the situation in the sheep uterus). We will hysterectomize sheep, and isolate and culture their uterine cells. We will test the cultured cells for up-regulation of ER mRNA by stabilizing the ER mRNA in response to estrogen.

9802163 The Effect of Scrotal Insulation on Morphology and *In Vitro* Fertility of Bovine Spermatozoa

Parrish, J. J.
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Grant 98-35203-6359; $95,851; 3 Years

The single largest factor limiting production of animal agriculture is fertility. Males, through the use of artificial insemination, have an impact on large numbers of females. It is therefore important to examine potential causes for reduced male fertility and how it might

be detected. To accomplish this we will examine the effects of scrotal insulation (simulates heat stress) on sperm morphology and *in vitro* fertility. Aim A is to examine how scrotal insulation effects the morphology of bull sperm. We will use a novel approach, Fourier harmonic analysis, that has been adapted by this laboratory to evaluate sperm shape through computer aided image analysis. In addition, the distribution of DNA within the sperm head will be determined using texture analysis of sperm digital images. Aim B will examine how scrotal insulation effects *in vitro* fertility. Sperm samples from both pre- and post-scrotal insulation will be used to determine if ability of sperm to initiate the fertilization process and sustain early embryo development has been affected. The direct application of this work is to the artificial insemination industry to improve methodology for semen quality exams. Long term, this research will provide the means for quantifying the effects of stressors on the production of sperm. The mechanisms of stress effects on sperm production and how to prevent it could then be addressed. This is important to the artificial insemination industry due to the increasing trend toward intensive housing of males and the inevitable increase in stress upon those males.

9801967 The Role of Endothelin in Ovarian Function: Follicular Development

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Strengthening Award; Grant 98-35203-6634; $160,000; 2 Years

The studies to be completed will enhance our understanding of how endothelin-1 (ET-1), a novel, locally produced ovarian factor, participates in the control of ovarian function. From recent studies, it appears that ET-1 is present and has direct actions in porcine, bovine, human and rat ovarian cells. However, the physiological role of ET-1 in the ovary has not yet been elucidated. Pigs will be used as a model for these studies. To begin to understand the physiological role of ET-1 in the ovary, the following supporting objectives are proposed. A study is proposed to identify the subtypes of ET receptor(s) contained within the porcine ovary. The knowledge gained in this experiment will allow study of the hormonal regulation of the receptor during the ovarian cycle, and it is a necessary step in elucidating the physiological role, if any, of ET-1 in the ovary. A second study is proposed to determine the follicular pool endowed with mature ET-1 peptide and mRNA, and its relationship to follicular atresia on specific days of the ovarian cycle. A third experiment will study follicular levels of estradiol, progesterone and ET-1 in follicles collected at specific days of the ovarian cycle. In summary, these studies will characterize the role of a novel ovarian modulator in the dramatic transition in granulosa cells from estrogen to progesterone production. Understanding the factors responsible for this transition may have important implications for the control and enhancement of fertility.

ANIMAL GROWTH, DEVELOPMENT, AND NUTRIENT UTILIZATION

Panel Manager - Dr. Douglas N. Foster, University of Minnesota
Program Director - Dr. Debora L. Hamernik

Research in this program area contributes to our understanding of the biological mechanisms underlying growth and development in agriculturally important animals. Emphasis is placed on innovative approaches in several research areas including but not limited to: cell proliferation and differentiation, genetic mechanisms underlying growth and development, metabolic regulators such as growth factors, synthesis and degradation of protein and lipid at the cellular or tissue level, metabolic and nutritional aspects of growth and development including rumen microfloral development and cellular and molecular aspects of the effect of environmental stress on growth and development. Development of dynamic modeling systems assessing specific quantitative estimates of nutrient requirements is also encouraged.

9803663 ASAS Biennial Growth Symposium: Current Concepts of Animal Growth IX

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Grant 98-35206-6040; $9,000; 1 Year

Funding will provide partial support for an international symposium on growth biology to be held in conjunction with the 1998 combined meetings of the American Society of Animal Sciences and American Society of Dairy Sciences. The symposium will begin with a keynote address on genetic and transgenic animal models to study growth. The morning session will be devoted to an emerging area of research, the role of the fat cell in growth, metabolism, and disease. The first talk will examine peroxisome proliferator activated receptor gamma (PPARg)-linked signal transduction mechanisms and will be followed by studies of PPARg and growth. The third topic is the role of C/EBPa in coordinating the activation of fat cell specific genes. The final topic for the morning session is the role of the fat cell hormone, leptin, in food intake, growth and obesity. The focus of the afternoon session is on novel concepts or novel methodologies to employ in growth research. The first speaker will examine nutritional regulation of downstream regions of the insulin-like growth factor-I (IGF-I) gene. This will be followed by studies on adrenomedullin, a recently discovered hormone found to regulate cellular growth and differentiation. The final topic of the symposium is a novel method for repeatedly examining gene expression in living cells. This symposium provides a unique mechanism for animal scientists to interact with leading biomedical scientists in the area of growth biology. The Biennial Growth Symposium has a history of fostering new research initiatives, new collaborations, and providing animal scientists with new and novel approaches to improving animal growth.

9801127 Muscle Satellite Cell Workshop: Growth, Regeneration & Muscle Disease

Allen, R. E.
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Grant 98-35206-6164; $10,000; 1 Year

The purpose of this conference is to bring together scientists from around the world who are conducting research on skeletal muscle satellite cells. Satellite cells are the postnatal muscle precursor cells that are responsible for normal muscle growth and development, muscle regeneration, and work-induced hypertrophy.

There has never been a conference devoted specifically to the biology of muscle satellite cells; research on these cells has been scattered throughout a variety of venues, with primary emphasis on cell biology, aging, neuromuscular disease, agriculture or exercise and sports science. As a result, there has been inadequate exchange of information and ideas among scientists that work on satellite cells. Progress in this field has undoubtedly been compromised by this situation.

The conference will be held August 13-16, 1998 in Boston, MA. Topics include a keynote lecture and workshop sessions. There will be an average of two invited speakers per session and the balance of each session will be composed of short, informal research presentations and discussions by participants. Sessions will center on the following topics: (1)origins and lineages of satellite cells, (2)cellular regulation of satellite cells, (3)role of satellite cells in normal muscle growth, (3)role of satellite cells in regenerating muscle, (5)activity of satellite cells in muscle adaptation, exercise and aging, and (5)activity of satellite cells in muscle disease and therapies. The conference will assemble new and established investigators from a variety of sectors within biology to exchange ideas, facilitate collaborations and identify significant new issues in satellite cell research.

9803665 Activation of Quiescent Skeletal Muscle Satellite Cells by Hepatocyte Growth Factor

Allen, R. E.
University of Arizona; Department of Nutritional Sciences; Tucson, Arizona 85721
Grant 98-35206-6840 ; $210,000; 3 Years

The long-range goal of this research is to understand the mechanisms underlying muscle growth in meat producing animals. Postnatal muscle fiber growth is dependent on the acquisition of new nuclei. This is accomplished by the division and fusion of satellite cells, the muscle precursor cells in postnatal muscle. Even though satellite cells actively divide and differentiate during growth, the population is not depleted, and most satellite cells are actually found in a quiescent state most of the time. Consequently, it is very important to understand how quiescent satellite cells are activated and stimulated to divide.

Research conducted on this project in recent years has identified hepatocyte growth factor (HGF) as the first factor known to be able to activate quiescent satellite cells. Hepatocyte growth factor, a protein with hormone-like activities, was able to activate quiescent satellite cells in culture assays and in living muscle. The hypothesis being tested is that satellite cell activation involves a dual signaling process, where HGF cooperates with another growth factor, insulin-like growth factor (IGF), to stimulate division of dormant satellite cells. The proposed project will integrate cell culture studies with experiments using living muscle from rats and cattle. The specific aims for this proposal are: 1) to investigate the role of the IGF system in satellite cell activation, 2) to investigate the interactions of growth factors in regulating satellite cell activation and muscle regeneration *in vivo*, and 3) to determine the effects of HGF on bovine satellite cell activities. A thorough understanding of growth factor regulation of these satellite cell activities will lead to possible strategies for enhancing muscle growth and repair.

9803619 Role of the Calpain System in Skeletal Muscle Development and Growth

Goll, D. E.
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Grant 98-35206-6646; $165,000; 3 Years

Growth of skeletal muscle ultimately depends on three things: 1)rate of muscle protein synthesis, 2)rate of muscle protein degradation, and 3)size and number of muscle cells. This project focuses on the second and third of these factors. The calpain system consists of two proteolytic enzymes, micromolar calpain (m-calpain) and millimolar calpain(m-calpain); a third protein that specifically inhibits activity of the two calpains and no other protease (calpastatin); and a fourth molecule that has been identified as a mRNA that exists only in skeletal muscle cells, that predicts a polypeptide with 51-55% sequence homology to m or m-calpain, respectively; and that has not been identified in its protein form (skm-calpain). The available evidence indicates that the calpains are responsible for initiating turnover of the myofibrillar proteins by specifically degrading those proteins that keep the myofibrillar proteins assembled in the myofibril; other proteases such as the proteasome are needed to degrade the myofibrillar proteins completely to amino acids. Disassembly from the myofibril may be the rate-limiting step in myofibrillar protein turnover, however. Calpain activity is also needed for myoblast fusion and evidence in nonmuscle systems has suggested that calpain activity is necessary for mitotic cell division. Hence, the calpains also have an important role in number and size of muscle cells. This project focuses on learning how activity of the calpain system is regulated in skeletal muscle cells, with emphasis on the roles of phosphorylation and calpastatin in this regulation, and in learning whether calpain activity is necessary for proliferation of myoblasts during muscle development.

9803723 Organization and Regulation of Chicken Fast Myosin Heavy Chain Genes

Bandman, E.
University of California, Davis; Department of Food Science and Technology; Davis, CA 95616-8598
Grant 98-35206-6395; $239,203; 3 Years

This proposal will describe how genes that produce the most abundant meat protein, myosin, are controlled during animal growth and development. Myosin is the major protein consumed in animal foods and its biochemical and biophysical properties are responsible for protein functionality in processed meats. An understanding of myosin gene regulation will provide important insights into the regulation of animal growth and development since myosin represents 50% of muscle mass. These studies will specifically target chicken myosin genes which have been shown to exhibit distinct properties from myosin genes in other animals. Chicken is not only an agriculturally important species, but has become the preferred choice of the meat consumer over the last decade. The results of these studies will ultimately provide new insights into the genetic mechanisms that control chicken muscle growth, and may also provide new tools with which to enhance muscle mass, improve lean meat yield, and increase the efficiency of meat production in food animal systems.

9803669 Unique Promoter Regulating Trophoblastic Differentiation

Conley, A. J.
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Grant 98-35206-6394; $185,000; 2 Years

Failure to establish or maintain pregnancy (days open), as well as fetal and subsequent neonatal growth retardation in livestock represent major sources of loss for the average producer. The long term goal of this project is to increase embryonic, fetal and neonatal growth and survival which is critical to the economy of livestock production. Successful pregnancy is absolutely dependent on the formation of a placenta, a failure of which causes embryonic mortality. Estrogen secretion is an early signal of placental development

and is essential for the establishment of pregnancy in pigs. We have identified a unique mechanism regulating the expression of genes necessary for estrogen synthesis in the developing placenta of the pig.
Other genes appear important in this process and may be equally important in other aspects of placental development and differentiation. This project will isolate at least one of these regulatory genes. These data will provide basic information on molecular mechanisms controlling the successful establishment of the placenta and pregnancy in pigs. We anticipate that the proposed research and future studies on the molecular control of this differentiation process will help to improve embryonic, fetal and subsequent post-natal growth and development in livestock species.

9803675 Gordon Research Conference on Mammary Gland Biology
Neville, M. C.; Daniel, C. W.
University of Colorado; Department of Physiology; Denver, CO 80262
Grant 98-35206-6468; $10,000; 1 Year

This grant will support the Fifteenth Biennial Gordon Research Conference on Mammary Gland Biology to be held at New England College, Henneker, New Hampshire, June 6 - 11, 1999. This conference brings together scientists from all communities with an interest in the Biology of the Mammary Gland: including Agricultural Scientists, Physiologists, Cell and Developmental Biologists and Cancer Biologists. The focus of the conference is the basic biology of mammary development and milk secretion. This year the major themes of the conference will be the role of steroid hormones, extracellular matrix and growth factors on mammary gland development, the function and evolution of milk protein genes and adaptations of lipid metabolism to lactation. Sixteen young investigators will be invited to present their work in poster discussion sessions. Workshops on new techniques will allow investigators to consider advanced technologies for their own laboratories. An understanding of these fundamental problems and new experimental technologies will aid the agricultural community in producing milk of a desired composition in the most cost effective manner.

9803641 Regulation of Teleost Growth Hormone Genes *In Vitro* and *In Vivo*
Chen, T. T.
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Strengthening Award; Grant 98-35206-6443; $200,000; 3 Years

In rainbow trout, two different genes exist which code for growth hormone (GH1 and GH2). In the regulatory region of the rainbow trout GH1 gene, analogous sequences, termed hormone response elements (HREs), have been identified based on similarities to several mammalian HREs. The differences between the type and number of the rainbow trout GH1 and GH2 gene HRE sequences underscores the need for studies aimed at determining the biological significance of these differences during rainbow trout development. Additionally, hormonal factors that regulate the expression of fish GH genes during development are not yet well defined. Our long-term goals are to understand: (i) the structure and function of the GH-family genes and their products, (ii) the regulatory mechanisms that control their gene expression, and (iii) the biological actions of these hormones at the cellular and molecular levels in teleost growth and development. To address these goals, we have chosen the economically important finfish, rainbow trout, as our *in vivo* model and a trout pituitary cell line (RTP-2 cells) as our *in vitro* experimental system. We have developed a rainbow trout pituitary cell line (RTP-2) that expresses the GH family genes, growth hormone (GH), prolactin (PRL) and somatolactin (SL). Preliminary studies support the hypothesis that other hormonal factors regulate the expression of GH genes in this cell line. Since we have elucidated the structure of fish GH genes and developed a continuous pituitary cell line, we can now investigate the molecular mechanisms controlling GH gene expression in teleosts and begin to characterize/identify hormones/factors which stimulate the GH gene in teleosts. A complete understanding of the role of the GH family of polypeptides and their genes during teleost development, growth and reproduction is a basic requirement before we can use the strategies of biotechnology to enhance aquaculture production.

9803644 Genes and Regulatory Mechanisms Controlling Growth Hormone Action in Chickens
Burnside, J.
University of Delaware; Department of Animal and Food Sciences; Newark, DE 19717
Grant 98-35206-6595; $185,000; 2 Years

Growth hormone (GH) has been used effectively to alter body composition and milk production of cattle. These improvements in GH-treated animals are linked to up-regulation of the GH receptor, increases in plasma insulin-like growth factor-I and a repartitioning of nutrients into lean muscle (or milk) instead of adipose tissue. However, unlike mammals, young, rapidly growing broiler chickens appear to be resistant to treatment with GH, despite the fact that their normal growth is GH dependent. We hypothesize that the positive effects of elevated GH are limited in chickens by negative regulation of the intracellular signaling pathway by GH. The recently described suppressors of cytokine signaling (SOCS) genes are likely candidates for mediating the negative effects of GH in chickens. These genes are transiently induced by ligand and block receptor signaling, thereby forming a simple negative feedback loop. We propose to study the role of SOCS in GH signaling in chickens. To complement these studies on the molecular mechanism of GH action in chickens, we will identify other GH-regulated genes as possible targets for therapeutic or genetic manipulation of growth. This will be approached by use of high throughput sequencing and DNA microarray technology. The overall results of this study will help define the molecular

basis of growth hormone (GH) action in chickens. Improved efficiency in the production of poultry depend on optimization of growth parameters and this requires a detailed understanding of the molecular mechanisms involved in GH and action.

9803625 Growth Regulation in a Warm Water Teleost, Tilapia (*Oreochromis mossambicus*)
Grau, E. G.
University of Hawaii; Hawaii Institute of Marine Biology; Kaneohe, HI 96744
Grant 98-35206-6444; $155,000; 3 Years

The goal of our research is to learn how growth and development are regulated in an important aquaculture species, the tilapia, *Oreochromis mossambicus*. We have found that tilapia grow 2-3 times faster when reared in seawater (SW) or treated with 17 alpha-methyltestosterone (MT). Fish grow 5-7 times faster when these treatments are combined. The enhancement of growth by SW and MT occurs mainly during the period of growth up to 60g. Our identification of distinct periods and situations in which growth is enhanced provides a special opportunity to study how growth may be regulated. We will determine whether discontinuance of MT treatment at 60g reduces subsequent growth. We also propose to characterize the effects of MT on two hormones which regulate growth: growth hormone (GH) and insulin-like growth factor (IGF-I). Specifically, we shall examine the effects of MT treatment and its discontinuance at 60g on: 1)pituitary GH mRNA levels; 2)plasma GH levels; 3)GH release from the pituitary; 4)hepatic IGF-I levels; 5) sulfation activity in the blood, a measure of IGF-I activity; and 6)changes in IGF-binding proteins. We expect the results of these studies to provide new and important information for the development of new biotechnologies and husbandry strategies to enhance growth of tilapia and other fish by manipulating endogenous growth regulators. We believe that MT treatment can be discontinued when the animals reach 60g without a significant decline in growth. Since tilapia are typically marketed at 400-500 gm, this hypothesis holds great promise for tilapia producers. If MT treatment could be discontinued at 60g with retention of full growth enhancement, the extended period for MT clearance prior to marketing would likely facilitate the practical application of this growth promoter.

9803636 Development of Host-Microbiota Interactions in the Piglet Intestine
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University of Illinois, Urbana-Champaign; Department of Animal Sciences; Urbana, IL 61801
Grant 98-35206-6429; $165,000; 3 Years

The neonatal period presents a formidable challenge to the swine industry. Twelve to 30% of liveborn piglets do not reach weaning age and those that do rarely reach their growth potential resulting in substantial production losses. Although neonatal deaths and growth stasis reflect numerous interactions between the piglet and its new environment, delayed intestinal development resulting in enteric compromise is a major underlying cause. Our proposal focuses on the development of the two major tiers of intestinal defense-the normal gut microbiota and the mucosal immune system. Addressed is the working hypothesis that a parallel and interactive relationship exists between succession of the normal adherent microbiota and the development of epithelial cell immunocompetence in the piglet intestine. An interplay between the host and its resident microbiota likely involves a complex signaling program between the two partners. As yet, we do not understand mechanisms underlying this reciprocal signaling process. To address those questions, our proposal brings together multidisciplinary expertise that features accomplished backgrounds in immunology, gastrointestinal microbiology, and intestinal biology. Molecular probe technology that overcomes long-standing methodology limitations in gut microbial ecology will be used to investigate concurrently the spatial and temporal development of the resident gut microbiota and the molecular basis of host-microbe interactions at the epithelium. Our research will develop the theory and lay the groundwork for unique biotechnological strategies to regulate the development of mucosal defense in the intestine and thereby offer the swine industry novel means to stimulate pig growth from birth through the weaning transition.

9803661 An Immunological approach to Selective Targeting of Pig Beta-Adrenergic Receptors
Mills, S. E.; Bidwell, C. A.
Purdue University; Department of Animal Science; West Lafayette, IN 47907-1151
Grant 98-35206-6380; $165,000; 2 Years

Beta-adrenergic receptors are a family of proteins present on the surface of all cells and are responsible for the coordinated "fight or flight" response initiated by the hormone adrenalin. This receptor system also coordinates body composition because activation reduces carcass fat and increases carcass muscle protein while improving the cost efficiency of production in livestock. Agriculture has not taken advantage of this system and the objective of this research is to develop novel activators that are specific for each family member, thus permitting selective targeting of the desired receptor and response. Our lab has cloned the pig beta-adrenergic receptors and we will use specific sequences to screen a protein library for ligands that bind and activate the receptors. Specificity and potency for activation will be determined using the cloned pig receptors expressed in cell lines in culture. Peptides specific for each receptor subtype can then be tested in pigs to determine which subtypes are linked to growth processes. Subtype-specific ligands will be valuable tools for dissecting how beta-adrenergic receptors coordinate cell metabolism, and for generating function-selective agonists for commercial application.

9803722 Nutritional and Hormonal Regulation of Hepatic Lipid Metabolism in Neonatal Pigs
Odle, J.

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Grant 98-35205-6645; $165,000; 3 Years

Prenatal (embryo & fetal) piglet mortality averages 25% and an additional 15-20% of piglets born alive die will during the neonatal period. Thus, the number of piglets produced per litter may currently be only half of what it potentially could be. The economic impact of this loss is enormous. The cost is ultimately carried by the consumer in the price paid for pork and mandates research aimed at decreasing these death losses. This research has the goal of improving postnatal survival by furthering our knowledge of the adaptations in fat metabolism that accompany birth. In stark contrast to other mammalian neonates, piglets do not demonstrate elevated ketone body production (ketogenesis) despite high milk-fat intake. Ketone bodies play a pivotal role in the transition from carbohydrate-based fetal metabolism to fat-based metabolism of the suckling newborn and provide an important alternative energy source for glucose-dependent tissues. Impaired adaptation would compromise the piglets ability to effectively utilize milk fat which in turn would increase mortality. Unfortunately, the regulation of fatty acid oxidation and ketogenesis in piglets has not been extensively studied and thus will be the focus of this project. The primary goal of this research is to examine nutritional and hormonal factors affecting gene expression & activity of fat-metabolizing enzymes in piglet liver tissue. Insights gained into the control and regulation of fatty acid oxidation and ketogenesis in piglets may allow for future manipulation(s) that could improve the utilization of milk-based calories and thereby improve piglet survival.

9803652 Role of Somatostatins in Regulating Growth of Teleost Fish

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Strengthening Award; Grant 98-35206-6410; $150,000; 2 Years

Organismal growth integrates a number of biological processes that must be precisely coordinated. It is well established that the growth hormone (GH)-insulin-like growth factor I (IGF-I) axis is a central element in growth regulation of mammals and fish. Information about how this axis is modulated, however, is lacking and critical questions remain regarding the mechanisms by which nutritional state, or other conditions of insulin deficiency, influence growth. Using rainbow trout as a model system we have obtained preliminary information that somatostatins, a family of peptide hormones of diverse molecular structure and wide-spread function, may play a critical role in growth regulation and mediate nutritional influences. With this project, we will use the rainbow trout system to examine for the first time in any species the extrapituitary role of somatostatins in the regulation of growth. We will first establish which somatostatins may be important in the regulation of growth. We will then identify the various levels of the growth axis (e.g., GH binding, IGF expression, etc.) at which somatostatins exert their effects. This project will provide important new basic information concerning growth regulation in fish and will contribute to the development of new aquaculture biotechnologies.

9803686 Role of IGF and IGFBP-3 in Bovine Mammary Epithelial Cell Growth

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Grant 98-35206-6428; $125,000; 2 Years

Insulin-like growth factor-I (IGF-I) is a peptide growth factor which regulates growth of the mammary gland. It also mediates a portion of the ability of bovine somatotropin to increase milk production in dairy cows. IGF-I associates with high affinity binding proteins (IGFBP) which modulate its biological activity. The mechanisms by which IGF-I and IGFBP regulate growth of the cells in the mammary gland which secrete milk are unknown. The MAC-T cell line represents a model to study such mechanisms *in vitro*. Exposure to IGF-I specifically stimulates IGFBP-3 synthesis by MAC-T cells. In addition, MAC-T cells that have been genetically altered to synthesize increased amounts of IGFBP-3 are more responsive to IGF-I compared to cells which do not synthesize IGFBP-3 under unstimulated conditions. These findings suggest a role for IGFBP-3 in enhancing IGF-I activity. In objective 1, the ability of IGF-I to stimulate the growth of MAC-T cells under conditions which alter synthesis of either IGFBP-2 or IGFBP-3 will be determined. Objective 2 will identify mechanisms which regulate IGFBP-3 gene expression in mammary cells. Both IGF-I and cAMP increase IGFBP-3 mRNA levels. However, neither factor alters the degradation of IGFBP-3 mRNA. Therefore, the ability of these factors to alter IGFBP-3 transcription rates will be determined. Understanding the basic biology of lactation physiology will allow us to utilize technological advances to (1)increase the length of time a cow lactates at a productive level before being rebred and (2)increase the efficiency of milk production. Such advances will not only improve animal well-being, but allow more milk to be produced from fewer resources which is essential to the sustainability of dairy farming in the United States.

9803693 Embryonic and Utero-Placental Expression of Pig CTGF

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Grant 98-35206-6430; $190,000; 3 Years

Failure of embryos to develop to birth is a significant problem in commercial animal production. In the pig, a high incidence of embryonic loss occurs within the first 30 days of pregnancy. Since it is known that polypeptide growth factors play a role in uterine, placental, and embryonic growth, the goal of this project is to determine the function of connective tissue growth factor (CTGF) in these processes. CTGF is a recently described molecule that stimulates cell proliferation, migration, and extracellular matrix production. We

have previously shown that CTGF is abundant in uterine luminal fluids of pigs where its levels are enhanced around day 11 of pregnancy. In addition CTGF is a mitogen for pig uterine stromal cells. Collectively, these observations support a role for CTGF in prenatal development of the pig. In several other systems, CTGF production is regulated by transforming growth factor-beta (TGF-b) suggesting that biological effects of TGF-b may be due to its stimulation and the subsequent action of CTGF. This project involves determining the localization of CTGF, TGF-b, and their cognate receptors in uterine, placental and embryonic tissues on days 0 - 30 of pregnancy and (ii) establishing the effects of TGF-β on CTGF production in cultured pig uterine stromal and epithelial cells and blastocyst trophectoderm cells. The outcome of this project will be to define mechanisms of cell growth control during early development in a species that is of commercial relevance to US agriculture but that is reproductively inefficient.

9801125 Research Conference Application: 1998 ASAS Growth and Development Program

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Grant 98-35206-5960; $5,882; 1 Year

The proposal requests partial support for the combined meetings of the American Society of Animal Science (ASAS) and the American Dairy Science Association (ADSA) to be held in Denver, Colorado on July 28-31, 1998. Three thousand scientists are anticipated to attend the 1998 ASAS/ADSA meetings. The program is designed to interface with other themes presented at the combined meetings by sharing symposia and by identifying commonalities between growth and lactation biologists. The program also features two presentations by scientists from corporate settings regarding recent research with Rismorelin, a potent, synthetic growth hormone secretagogue. Four symposia with eighteen speakers have been organized and include: 1) Muscle Architecture, 2) Cytokines and Animal Growth, 3) Advances in Transgenic Technology, and 4) Molecular Mechanisms of Hormone Action. A symposium on muscle architecture has not been included in the ASAS program for many years; thus, this topic is timely. The role of cytokines in animal growth is a rapidly emerging field of research. This symposium will focus on leptin, growth differentiation factor-8 (myostatin), interleukin-15, and insulin-like growth factor-1. The third symposium will focus on techniques for studying gene regulation in domestic animals and will include discussions of transgenic and embryonic stem cell technology, commercial opportunities for cloned animals and recent advances in gene therapy. The symposium on molecular mechanisms of hormone action will include presentations on growth hormone signaling, insulin action, adipocyte differentiation and functions of Rismorelin. These symposia are expected to form the core of the Animal Growth and Development Program at the 1998 ASAS/ADSA combined meetings.

9803674 Function of COUP-TF in Adipocyte Differentiation

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Grant 98-35206-6311; $125,000; 2 Years

Our knowledge regarding the biology of fat cell proliferation and differentiation remains incomplete. This lack of understanding is an obstacle for devising new methods to reduce the body fat content in meat producing animals. A great many papers have provided invaluable information regarding fat cell metabolism, development, and differentiation. However, the mechanisms regulating the differentiation of precursor fat cells to mature fat cells have remained largely unknown. A transcription factor called COUP-TF plays a key regulatory role in many developmental systems. We have shown that inhibition of precursor fat cell differentiation by treatment with dioxin or retinoic acid induces COUP-TF to bind more readily to the DNA of a fat cell specific gene. We have also shown that increased amounts of COUP-TF in precursor fat cells inhibits differentiation. The goal of this proposal is to extend our preliminary observations to the molecular level by examining the mechanisms by which COUP-TF inhibits precursor fat cells to differentiate. Our proposal presents a scheme for determining the function of COUP-TF in the differentiation of fat cells. This will be accomplished by using tissue culture cells which have COUP-TF DNA introduced into them. The time when COUP-TF protein is effective in this inhibition will be determined. Assays to characterize changes in RNA and protein will establish what changes occur to the factors, known to play a role in fat cell differentiation, with increased COUP-TF in the cell. This study will be the first to assess the function of COUP-TF in fat cell differentiation and will identify an important control point. This knowledge should afford a new means of controlling unwanted fat accretion in meat producing animals.

9803653 4th International Workshop on Lactation in Farm Animals

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Grant 98-35206-6048; $2,500; 1 Year

The Fourth International Workshop on Biology of Lactation in Farm Animals will be held on July 26, 1998 in Denver, CO in association with the combined annual meetings of the American Dairy Science Association and the American Society of Animal Science. This workshop convenes researchers, teachers, government and industry representatives to discuss new and exciting topics related to lactation in farm animals. The workshop includes the following sessions: 1)The Mammary Gland as a Bioreactor, 2)Variations in Normal Mammary Gland Function, 3)Local Regulation of Mammary Gland Function, and 4)Nutrition and Management Effects on Milk Production. Two invited speakers (nationally and internationally recognized for their contributions in their respective areas of research)

will provide presentations in each session. A third presentation in each session (of shorter duration) will also be selected from abstracts submitted to this program. Speakers and discussion leaders are encouraged to be thought-provoking and to encourage audience participation in the discussion. Proceedings of the workshop will be published as a supplement to the *Journal of Animal Science.* This workshop provides a unique forum for discussing state-of-the-art research involving lactation in farm animals.

9803668 Characterization of Phosphoregulatory Mechanisms Governing Avian Myogenesis

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New Investigator Award; Grant 98-35206-6284; $140,000; 2 Years

It has been reported that within the next forty years, the world must more than double its consumable foodstuff production to meet population demands. This is a daunting task, as it will require us to improve our efficiency of production while maintaining a watchful eye to environmental and health concerns. To enhance the efficiency of lean tissue deposition in meat-producing animals, our understanding of the molecular mechanisms responsible for skeletal muscle growth and development must be advanced. The primary research goal of our laboratory is the characterization of intracellular signaling events that alter the transcriptional mechanisms responsible for muscle-specific gene action. Currently, a large body of evidence exists delineating the absolute requirement of the myogenic regulatory factors (MRFs) for the proper formation and function of skeletal muscle. However, additional cofactors, including E47, MLP and MEF2, also play integral roles in dictating MRF-mediated gene transcription. We are examining E47 phosphorylation status as a myogenic regulatory mechanism. The proposed work involves the identification and characterization of specific phosphoregulatory sites within E47, which alter the protein's ability to form heterodimers with MRF family members. By construction of chimeric and deletion mutants of E47, we will address the functional impact of phosphorylated E47 during specific stages of myogenesis. Additionally, using a retroviral mis-expression system, we propose to examine the effects of altered E47 activity during avian myogenesis *in ovo*. The results generated from this proposal will play a pivot role in the design of future methods of enhancing skeletal myogenesis through modifications of the existing cellular machinery.

9803615 Growth Hormone-JAK2 Signalling in Chickens: Pathways for Muscle Growth

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Grant 98-35206-6411; $165,000; 2 Years

Growth hormone (GH) is recognized as a major regulator of postnatal growth and metabolism, yet until quite recently, nothing was known about the pathway by which this important growth factor communicated with target cells. Several components of the pathway are now identified, including formation of a complex between GH and specific recognition sites (GH-R) on the cell, association of an intracellular enzyme (JAK2 kinase) with this complex, biochemical changes (tyrosyl phosphorylation) in both the GH-R and JAK2, and downstream events involving activation and binding of proteins (STATs) to the nucleus of the cell, which effect changes in expression of target genes. Despite indications of a central role for JAK2 in GH-induced cellular events, direct dependency on JAK2 has not been demonstrated. During posthatch development, GH administration can elicit a variety of biological responses in poultry, but enhanced muscle growth, as occurs in mammals, is not realized. The pathway of GH signalling in avian cells has not been explored, and lack of a muscle response to GH in poultry may reflect tissue-specific differences in post-receptor signalling. The long-term goal of this project is to further define the mechanisms involved in GH signal transduction, including direct demonstration of the importance of JAK2, and to determine if tissue specific differences in post-receptor signalling events exist. Understanding these mechanisms is crucial for refining GH-based growth enhancers and therapies. Given the range of actions of GH, this research has relevance to animal agriculture.

9803616 Mechanism of Muscle Growth Stimulation by Somatotropin in Pigs

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Grant 98-35206-3657; $140,000; 2 Years

It is important that meat producers find ways to increase the amount of meat they produce and to lower feeding costs. This can be accomplished by treating pigs with the hormone, somatotropin, which increases muscle mass, and at the same time, lowers the amount of food eaten. We have recently found that treatment of pigs with somatotropin increases growth primarily by decreasing the breakdown of protein in the body rather than by stimulating the amount of protein synthesized. In addition, this stimulation of growth by somatotropin treatment causes a reduction in the catabolism of amino acids, which are the building blocks of protein. We propose to determine whether the reduction in protein breakdown occurs in all tissues of the body, including skeletal muscle. To do this, we will trace the movement of amino acids into and out of the proteins in different tissues of the body of pigs treated with somatotropin and control pigs. We also propose to determine whether the reduction in amino acid catabolism with somatotropin treatment is a direct effect of somatotropin on the enzymes which regulate amino acid catabolism. To do this, the activity of these enzymes will be determined in normal pigs and those treated with somatotropin. These studies will be of potential use to swine producers by identifying the way that somatotropin promotes muscle growth.

9803725 Nutritional Regulation of Mucosal Essential Amino Acid Catabolism

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Grant 98-35206-6310; $155,000; 2 Years

Quantitative information on the factors that affect the availability of dietary nutrients has the potential to significantly impact the economic efficiency of farm animal production. Recent research in pigs has suggested that the degradation of dietary amino acids by the intestinal tissues is an important source of inefficiency in protein utilization by growing animals.

The aims of this proposal are: (1) to quantify the effect of dietary protein intake on the role of nutritionally essential amino acids as an energy source for the intestine and (2) to study the effect of dietary lysine on intestinal lysine metabolism. The research will concentrate on the amino acids lysine and threonine because these are often the first- and second-limiting amino acids in conventional pig diets. The research will use amino acids labeled with the stable (NON-radioactive) isotope ^{13}C. These will be given to weaned piglets that have been implanted with a catheter in the vein that drains the intestine, so that net amino acid absorption and gut metabolism can be measured directly. In the first study, piglets will be given diets that support either maximum protein deposition, maximum efficiency of protein utilization or maintain the animals at protein equilibrium. We anticipate that the intestine will show little adaptation to total protein intake. In the second study, pigs will receive diets that are strongly or marginally deficient in lysine. In contrast to total protein intake, we anticipate that changes in lysine status will lead to an adaptation in intestinal lysine metabolism. The results will, we believe, add important information on nutritional factors affecting feed utilization and improve the accuracy of current protein-feeding standards for young pigs.

9803172 Adipogenesis in Pigs Fed Conjugated Linoleic Acid

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Grant 98-35206-6286; $68,000; 2 Years

Suboptimal growth and development limit livestock productivity, and basic information regarding the regulation of the production of fat in all livestock species is lacking. We will test the hypothesis that the naturally occurring fatty acid, conjugated linoleic acid (CLA) causes overexpression of grow factors that depress fat cell division. Conjugated linoleic acid may depress fat cell division in piglets, which could lead to a permanent reduction in the rate of fat accumulation. This research will be composed of three phases. The first will involve measuring the expression of genes encoding specific growth factors during the phases of division and lipid filling of cultured fat cells treated with CLA. The second will be to demonstrate the effects of feeding CLA to postweaning piglets on the division and lipid filling of fat cells located within abdominal and subcutaneous fat depots. Finally, the expression of the growth factor genes will be measured in fat depots from control and CLA-fed pigs to determine if this process is affected by CLA. There are two novel aspects for the proposed research: 1) we propose to demonstrate the efficacy of treating with CLA early in the life cycle, thereby reducing the amount of CLA necessary and increasing the ease of application; and 2) this research will provide one of the few opportunities in a livestock species to directly manipulate the expression of genes known to have profound effects on fat cells in culture. If CLA causes a permanent reduction in fat growth when administered to piglets, producers will realize increased conversion of feedstuffs to lean tissues, and consumers will be provided a leaner pork product.

9803664 Parenchymal and Stromal Modulation of the Local IGF-I Axis For Heifer Mammary Growth

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Grant 98-35206-6285; $150,000; 3 Years

The focus of our proposal is to understand the relevance of local mammary tissue production of insulin-like growth factor one (IGF-I) and the binding protein (IGFBP-3) in stimulation of mammary growth. We will test the idea that duct development is tightly regulated by changes in local tissue expression of these molecules. It is likely that increased local mammary tissue production of IGF-I explains the rapid allometric increase in mammary duct growth which begins prior to puberty. pecifically, we will test a hypothesis that communication between the mammary stromal and mammary ducts is essential for increased production of IGF-I for allometric mammary growth. his will be done by measurement of stromal tissue synthesis and secretion of IGF-I and IGFBP-3 in heifers in which the epithelial tissue is removed shortly after birth (a cleared mammary gland). Bioassay of stromal tissue extracts from intact and cleared mammary glands will be used to provide an additional direct test of our hypothesis. Companion cultures with added excess antibody to IGF-I will be used to determine the degree to which changes in concentrations of biologically active IGF-I is responsible for growth effects of the two types of stromal tissue extracts. Specific effects of exogenous growth hormone on expression of IGF-I and IGFBP-3 and cell proliferation will also be determined in stromal explants as well as for mammary parenchymal tissue from intact mammary glands. These data will increase our understanding of the role of the local IGF-I axis in mammary development and suggest possibilities for enhanced development.

9803717 Reciprocal Regulation of Fat and Muscle Mass by Interleukin-15: A Transgenic Model

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Grant 98-35206-6463; $200,000; 3 Years

A major goal of meat animal agriculture is to produce animals with increased muscle mass and decreased fat mass. This would result in healthier meat products (due to decreased fat content) and would also increase the efficiency of meat production (by targeting growth towards lean tissue accretion). We have shown that a recently discovered cytokine which is highly expressed in muscle tissue, interleukin-15 (IL-15), causes increased protein accretion when added to skeletal muscle cell cultures from mice and cattle. Additionally, treatment of laboratory rats with IL-15 for 1 week significantly decreased fat deposition. The current project will attempt to use molecular genetic engineering techniques to produce transgenic mice in which IL-15 is overexpressed in muscle tissue only, although we expect the factor to be secreted by muscle and carried to fat tissue by the circulation. If successful, we will then analyze the muscle and fat content of these mice compared to control mice, and analyze other parameters of importance in meat production (hormone levels, protein synthesis and degradation rates, etc.). These experiments seek to determine in an inexpensive laboratory animal if IL-15 can be utilized in growing animals to alter lean:fat ratios.

9803711 Regulation of Transcription of the Avian Gene for Acetyl-CoA Carboxylase

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Strengthening Award; Grant 98-35206-6467; $150,000; 2 Years

For at least three decades, the American public has been encouraged to decrease their consumption of fat and to increase the ratio of polyunsaturated to saturated fatty acids in the diet. These preventative health recommendations stem from a correlation between saturated fat intake, serum lipids, and the risk of heart disease. A metabolic process that plays an important role in controlling the fat content of poultry and other domestic livestock is the pathway for the synthesis of long-chain fatty acids. Acetyl-CoA carboxylase is an enzyme that catalyzes the pace-setting step of the fatty acid synthesis pathway. The level of this enzyme is regulated by changes in nutritional status. Little is known about the mechanisms involved in the regulation of acetyl-CoA carboxylase expression in the chicken. The long-term objective of this proposal is to understand the molecular basis for nutrient- and hormone-induced changes in acetyl-CoA carboxylase expression in chick liver cells. Our experimental approach will be to identify sequences in the acetyl-CoA carboxylase gene that confer the effects of nutrients and hormones on acetyl-CoA carboxylase expression. Data from studies will be used to develop new strategies to inhibit the synthesis and accumulation of fat in chickens.

ANIMAL GENETIC MECHANISMS AND GENE MAPPING

Panel Manager - Dr. Jerry B.Dodgson, Michigan State University, East Lansing
Program Director - Dr. Peter R. Brayton

The objective of this program is to increase our knowledge and understanding of the structure, organization, function, regulation and expression of genes in agriculturally important animals including aquaculture species. This includes but is not limited to: comparative gene mapping and the identification, isolation, characterization of genes, gene products and their regulatory mechanisms, identification and mapping of DNA segregation markers including quantitative trait loci (QTL), interactions between nuclear and organellar genes and the molecular basis of genetic replication, and development and application of methods to modify the animal genome.

9803490 Linkage Mapping of Quantitative Trait Loci in Catfish

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Grant 98-35205-6738; $236,000; 3 Years

Channel catfish is the most important cultured fish in the US accounting for over 50% of all US aquaculture production. A sustainable catfish industry, however, relies on improved brood stocks. Several major threats to the catfish industry need to be urgently addressed including disease resistance, growth, feed conversion efficiency, and carcass composition. Because most economic traits are polygenic and quantitative, understanding genomic organization and QTL mapping is required for breeding programs with marker-assisted selection, for genetic improvements through biotechnology, and for introducing beneficial genes from related species such as from blue catfish. As a first step of catfish gene mapping, our specific objective is to generate a catfish genetic linkage map with 5-10 cM resolution and to scan the catfish genome for quantitative trait loci (QTL) controlling growth rate and body conformation. Growth rate is of top priority because it affects both production efficiency and profitability. Body conformation is a strong indicator of carcass yield. The channel-blue catfish hybrid system offers tremendous marker and phenotypic variations. The high fecundity of catfish allows use of full-*sib* families and effective phenotypic selection for detection of QTLs. QTL-linked markers generated from this project will be highly useful for breeding programs. Improvement growth and carcass composition in catfish will increase production, efficiency, profitability and sustainability of catfish aquaculture, which, in turn, should increase catfish exports, reduce the US seafood trade deficit, reduce pressure on natural fisheries, and enhance environmental quality.

9803463 High Resolution Mapping of the Sex Chromosome in Rainbow Trout

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Postdoctoral Fellowship; Grant 98-35205-6642; $90,000; 2 Years

Rainbow trout (*Oncorhynchus mykiss*) are one of the most economically important aquaculture species in the United States. They possess an X/Y system of genetic sex determination consisting of XX females and XY males. In contrast to mammals, where the majority of the Y chromosome has lost the ability to recombine with the X chromosome, the X and Y chromosomes of rainbow trout have only slightly differentiated. I propose to produce a high resolution genetic map of the rainbow trout sex chromosomes using AFLP markers and bulked segregant analysis, a DNA pooling strategy capable of rapidly identifying markers near a trait of interest. Markers will be genetically mapped in two androgenetically-derived doubled haploid panels of rainbow trout produced from populations that contain morphologically distinct Y chromosomes. This strategy should result in the identification of a male-specific marker on the Y chromosome. In addition, comparing the genetic structure of morphologically distinct Y chromosomes will determine the structural changes associated with the evolution of morphologically distinct sex chromosomes in this species. Finally, I will attempt to identify and map rainbow trout sex-linked markers in coho salmon and Atlantic salmon in order to assess the homology of the sex chromosomes in these closely related species. The identification of a Y chromosome-specific molecular marker will make it possible to determine the sex of immature fish which should greatly benefit the aquaculture industry. Additionally, this research will enhance the existing genetic map and increase our knowledge of sex chromosome evolution and genome organization in vertebrates.

9803441 Rfp-Y Genes and the Response of Chickens to Infectious Disease

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Grant 98-35205-6643; $200,000; 2 Years

Controlled immune responses are important in fighting infections in poultry. Infectious organisms are recognized and destroyed by specialized cells of the immune system. Precise molecular interactions make the necessary responses highly specific. One class of molecules especially important for this are those which display bits of viral protein (peptide) on the surfaces of virally infected cells. Rfp-Y is a region in the genome of the chicken discovered recently through DNA analyses. Genes within Rfp-Y may provide molecules

of this class and hence be an additional means of controlling the precision with which immune responses are made. The aim of this project is to determine whether the newly recognized Rfp-Y genes are indeed producing molecules capable of directing immune responses at sites of viral infection. Additional experiments are directed toward elucidating genetic variability at the Rfp-Y loci so that particular alleles may be studied for their effectiveness in directing immune responses. These basic studies may eventually contribute a means of enhancing the immunity of poultry stock to a variety of viral diseases of economic importance including Marek's disease and avian leukosis.

9803448 Use of DNA Microarray to Analyze Gene Expression Patterns During Avian T-Cell Development

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Grant 98-35205-6640; $220,000; 3 Years

Controlling disease is a major challenge for the poultry industry. New pathogens and highly virulent strains of known pathogens escape current vaccination protocols. In order to meet this challenge in the future, a better understanding of the biology of the immune system is needed. We have sequenced over 3000 expressed sequence tags (ESTs), including over 1000 novel genes, from chicken immune tissue. We will use these sequences and recently developed cDNA microarray technology to simultaneously examine changes in gene expression in the immune system, during early development of immunocompetence and during the course of infection with Marek's disease virus. These studies will enhance our understanding of the molecular events mediating normal immune cell development and activation in response to disease. The results will reveal whether the acquisition of immunocompetence is related to developmental changes in expression of particular genes (known or novel) in immune tissues. The long terms goals are to be able to manipulate the chickens immune system by either gene therapy or by administration of natural immune modulators that accelerate the maturation of the immune system.

9803446 Structure and Regulation of the Porcine Aromatase Gene Family

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Grant 98-35205-6739; $210,000; 2 Years

In the pig, the signals for alterations in uterine activity necessary for establishment of pregnancy are the estrogens transiently synthesized at high levels by the aromatase enzyme in late preimplantation stage embryos. We have recently identified a novel form of aromatase enzyme responsible for the bulk if not all, of the estrogen biosynthesis by the periimplantation pig embryo. This protein is structurally distinct from two other pig aromatase isoforms, one of which is expressed m endometrium and placenta and the other which is expressed in ovary, fetal adrenal and fetal testis. Our data suggest that the generation of these distinct aromatase isoforms is due to multiple aromatase chromosomal genes possibly comprising a closely-linked gene cluster. Elucidation of the molecular mechanisms responsible for aromatase isoform-specific protein synthesis requires the isolation, characterization and functional examination of the corresponding chromosomal genes. The specific aims of this proposal are to: a) isolate and characterize the gene promoter and 5'-flanking regions of the aromatase genes; b) examine the structural basis for tissue-specific expression of these genes; and c) elucidate the potential regulation of embryo aromatase gene expression by a uterine cytokine (leukemia inhibitory factor, LIF) transiently expressed at Day 12 and previously implicated in human and mouse implantation events. Such studies should provide insights into the biological basis for aromatase gene expression and may lead to development of new biotechnology-based strategies to select for enhanced embryo survival and subsequent fetal development in agriculturally important animals.

9803480 Radiation Hybrid Mapping of Cattle ESTs for High Resolution Comparative Genomics

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Grant 98-35205-6644; $250,000; 2 Years

Isolation of genes controlling economically important traits and functional characterization of their products will require a dramatic increase in the number of coding genes on the cattle gene map. Towards this end, we propose to identify 1500 genes expressed by cattle ovary tissue and map 500 of these genes using a powerful and unique new resource for radiation hybrid mapping. This will nearly double the number of known and ordered genes on the cattle gene map. Ovary was selected because it is a rich source of transcripts involved in reproduction and development, and may thus be a valuable source of genes affecting fertility traits. Mapped genes will be used for building detailed comparative genome maps, understanding genome evolution and leading the way towards molecular identification of genes affecting economically important traits. Realization of the long-term objectives of this research will enhance the competitiveness of U.S. dairy and beef industries in national and international markets via improved efficiency of production systems and yield of quality products.

9803437 Development and Testing of Fish Transgenic for the Phytase Gene

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Grant 98-35205-6762; $148,000; 2 Years

Phosphorous is required by all living organisms to sustain life. Availability of Phosphorous in the environment is very limited, and is thus a precious commodity for growth in both the plant and animal kingdoms. Because phosphorous is limited, plants package phosphorous directly in seeds to provide all critical nutrients necessary for growth. However, to protect phosphorous from being consumed by other organisms, plants store phosphorous in a special form called phytic acid. In this form, animals cannot digest the nutrient. As a result farmers lose money because inorganic phosphorus must be added to the diet and is expensive. The environment suffers because the majority of phytate phosphorous is excreted in manure. Unfortunately there are several microorganisms which can breakdown phytate into its elemental form by use of an enzyme called phytase. When elemental phosphorous runs into lakes or streams, algae blooms results, which deplete the water of oxygen and result in massive fish kills and cascading effects on other life forms. A solution to this problem is proposed in this project that results in a win-win situations for the farmer and the environment. We propose to transfer the gene that codes for phytase from microorganisms directly to animals. Specifically we plan to construct a gene which expresses in the digestive system, test the gene in cell culture for expression, and produce transgenic fish (medaka) with those constructs.

9803475 Optimizing Marker-Assisted Selection for Genetic Improvement of Livestock

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Grant 98-35205-6736; $130,000; 3 Years

Ongoing molecular genetic research into the genetic analysis of important traits in livestock, is uncovering genes and genetic markers that can be used to enhance selection of improved breeding stock through marker-assisted selection. Knowledge on how to use these identified genes to enhance genetic improvement of livestock in a sustainable manner is, however, not well developed. This lack of knowledge was recently demonstrated by several computer simulation studies, which found that current strategies for marker-assisted selection can jeopardize genetic improvement over the longer term. The main objective of this research is, therefore, to develop selection strategies for marker-assisted selection that optimize the utilization of identified genes in genetic improvement programs for livestock. Advanced mathematical methods will be used to develop such selection and breeding strategies. As a feasible alternative to extensive animal experimentation, computer simulation models of livestock breeding programs will be used to test the effectiveness of the new selection strategies. The outcome of this research will be enhanced strategies for marker-assisted selection for a range of livestock breeding programs. This will be of importance to U.S. agriculture because these enhanced strategies will allow greater benefits to be obtained from advances in molecular genetics. Because the emphasis in this research is on the better utilization of molecular genetic information that is generated by ongoing molecular genetic research, little additional costs will be needed for the US agriculture to capitalize on the benefits of the enhanced strategies that will be developed herein.

9803483 First International Pig Chromosome 13 Workshop

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Grant 98-35205-6906; $2,000; 1 Year

Genetic loci controlling important traits in the pig have been identified on chromosome 13 (SSC13), including traits for growth, carcass characteristics, and bacterial infection of piglets. However, the actual genes have not been identified. Several collaborative groups have focused substantial efforts on SSC13 to understand and pinpoint the genes affecting these economically important traits. These joint projects have progressed to the stage that a public forum for discussion would be highly useful for further progress to be made. Speakers at the Workshop will report unpublished work on newly combined genetic and physical maps for SSC13, current progress in comparative mapping, and the current status of projects to map the location of growth and disease resistance traits on SSC13. The sponsorship by the USDA of this Workshop is directly related to the mission of the USDA in increasing the information available on the genomes of animals, developing the means to improve the characteristics of livestock, and increasing the global competitiveness of US agriculture. As several of the projects and new technologies to be presented and discussed at the Workshop are being performed outside the US, the Workshop will be an opportunity for US researchers to learn up-to-date technologies which can further research in mapping projects of interest to US agriculture. Thus a final important Workshop activity will be discussion of additional collaborative projects to further global pig mapping research.

9803450 Identification of Quantitative Trait Loci for Production Traits in Poultry

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Grant 98-35205-6707; $235,000; 3 Years

At 41% and growing, poultry meat and eggs are the main meat consumed in the U.S., and the U.S. exports over $2.5 billion of poultry products. Primarily due to advanced breeding programs, tremendous progress in growth and egg production have been achieved to meet the demands of consumers. Modern genetics will compliment and extend the ability of the poultry industry to breed elite chickens

more quicker and more efficiently. This project will develop the basic diagnostic tools to select superior animals in commercial flocks. Using DNA markers, genetic tests for enhanced growth or egg-laying rates will be generated. Ultimately, the U.S. consumer will benefit by having access to safe and economical poultry products.

9803445 High Resolution Genetic Maps for Cattle and Sheep

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Grant 98-35205-6763; $220,000; 2 Years

Our goal is to rapidly integrate the genetic and physical genomic maps of cattle and sheep with markers transportable across species, populations, breeds and families. Our hypothesis is that high throughput scoring of markers that recognize identical locations on the chromosomes of cattle and sheep will rapidly 1) provide high-density framework maps into which markers developed through other technologies can be readily integrated, and 2) improve our ability to refine the chromosomal location of genes that account for significant effects on carcass quality, reproduction and disease susceptibility in any cattle or sheep population. Our strategy provides the additional, perhaps crucial, markers to scientists and breeders engaged in mapping the chromosomal location of economically important genes or using markers to assist their selection process who do not have the resources to produce additional markers themselves. We will achieve this goal using an international, multi-institutional, integrated strategy of marker isolation, characterization and mapping to reduce overall costs and improve efficiency. This approach will allow us to isolate, characterize and map approximately 2000 new markers on each of the sheep and cattle maps within three years. All map information will be provided to potential users through existing NAGRP distribution networks including the World Wide Web.

9803428 11th North American Colloquium on Domestic Animal Cytogenetics and Gene Mapping

Ponce de Leon, F.A.; Schook, L. B.; Beattie, C. W.
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Grant 98-35205-6905; $4,000; 1 Year

The central objective is to organize the 11th North American Colloquium on Domestic Animal Cytogenetics and Gene Mapping in Minneapolis-St. Paul, July l999. This is a colloquium that brings together researchers and students specialized in classical and molecular cytogenetics. Since the early seventies the contribution of cytogenetics to the development and saturation of genetic and physical maps for farm animal species has been significant. The organization of this colloquium seeks to stimulate and encourage the application of new molecular cytogenetic technological developments and to stimulate students to continue work in this area of research. The colloquium is seen as a forum for researchers and students to identify opportunities for collaborations and continued application of new technologies that enhance our understanding of the genome organization of farm animal species. Past meetings have attracted an average of seventy national and international participant of which about 40% are students.

9803470 Fine Mapping of a QTL Affecting Growth Rate on Swine Chromosome 1 (SSC1)

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Grant 96-35205-3546; $160,000; 2 Years

The goal of this research project is to improve our ability to use genetic markers to identify pigs with increased growth rates. The proposal represents an extension of a previous funded project which utilized an interdisciplinary approach and the University of Illinois Meishan x Yorkshire Resource Family to identify economically important traits in pigs. The strategy employed in this proposal allows for the microdissection of specific chromosomal areas of swine chromosome 1 which have been shown to contribute to over 25% of the average daily gain observed in the Meishan x Yorkshire breed cross. After the physical dissection of this chromosomal region is completed, large DNA inserts of the chromosome will be cloned. This will allow for the isolation of genes in that chromosomal region which may contribute to growth. A particularly unique aspect of this proposal is the use of a porcine radiation hybrid panel. This radiation hybrid panel was developed by the investigators at the University of Minnesota in collaboration with scientists at INRA-Tolouse, France and allows for higher resolution of physical distances between genes on a chromosome. The use of the radiation hybrid panel permits comparing genetic information associated with growth and development obtained in studies using mice and humans to this project. This research will provide important genetic markers to assist pork producers in developing seedstock with higher feed efficiency and increased weight gain. Interactions with pork producers to assist in the rapid transfer of this technology will also be initiated.

9803484 Improved Statistical Methods for Detecting QTL and Estimating Their Effects

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New Investigator Award; Grant 98-35205-6709; $75,000; 3 Years

Quantitative trait loci (QTL) are genes that affect quantitative characteristics of plants and animals. Examples include the genes affecting fruit weight in tomatoes, body fat in pigs, and blood pressure in humans. The goal of this project is to improve key statistical methods genetic researchers use to detect QTL and estimate their effects. The first phase of the proposed research is related to the

detection of QTL through a popular computer-intensive technique known as permutation testing. A plan to measure the effect of uncertainty introduced by permutation testing and to reduce its impact on the conclusions of QTL analyses is developed. The second phase of the research is focused on estimating the genetic effects of identified QTL. Estimation of the genetic effect for a given position is accomplished while directly controlling for the effects of all other detected QTL. Such analyses provide insight into the type of gene action at each QTL and can be used to answer many natural questions that arise once multiple QTL have been identified. The methods developed will maximize the benefits of QTL data by improving the quality of the inference made from the data. Such improvements naturally lead to a deeper understanding of the relationship between locus and trait and, perhaps, to the development of organisms that are genetically superior to existing stocks with regard to valuable agronomic traits. Such developments will enhance the world-wide competitiveness of the United States agricultural industry by boosting productivity and efficiency at no additional environmental cost.

9803476 Mapping the Tilapia Genome

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Grant 98-35205-6639; $140,000; 2 Years

Aquaculture represents one of the fastest-growing sectors of the agricultural economy. In contrast to other agricultural species, most aquatic species are at a very early stage in domestication. Little or no genetic selection has been initiated for performance traits such as growth or disease resistance. This project extends our previous work on genetic mapping in tilapia (*Oreochromis niloticus*), a commercially important cultured finfish species. The genetic map for this species presently contains 163 polymorphisms, including 60 microsatellite markers, spanning a genome of approximately 1100 cM. In this project we will characterize an additional 160 microsatellite DNA loci from tilapia to provide markers with an average spacing of 7 cM across the genome. The chromosomal locations of these markers will be identified by assessing linkage in families of meiotic gynogens. The meiogyne family material will allow us to position loci relative to each other and to the centromeres of their respective chromosomes. Our ultimate goal is to use this linkage map to identify and select the genes controlling economically important traits in fishes.

9803477 Implementation of Marker Assisted Selection in U.S. Beef Cattle Breeds

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Grant 98-35205-6641; $235,000; 3 Years

In recent years, demand for beef has decreased, threatening the viability of the entire industry and increasing the importance of efficient production of a quality product. Independent research groups have been successful in localizing economic trait loci (ETLs) for carcass merit and growth, validating the utility of the gene mapping approach and indicating that the same genes are responsible for variation in quality attributes in many different breeds of cattle. Thus, marker-assisted selection (MAS) has the potential for widespread application in the multitude of breeds of beef cattle present in the U.S. today. The NCBA National Tenderness EPD project is aimed at integrating molecular markers for tenderness into EPD calculations in 1500 sires from 15 major beef cattle breeds and provides a $2.6 million dollar platform from which the utility of MAS for a large number of traits can be evaluated. The NCBA project will construct segregating families, obtain DNA samples and collect data for carcass traits. We will develop and score multiplex marker systems for eight growth and carcass merit ETLs, test the segregation of these ETLs in the NCBA families, identify favorable haplotypes for implementation of MAS within sire families, narrow each ETL region, and determine the persistence of haplotype/phenotype associations within each of the breeds. The overall objective of the proposed project is to evaluate previously identified ETLs for MAS in a wide variety of important US beef breeds. The primary outcome will be producer-ready marker tests with associated EPDs for growth and carcass traits.

9803455 Positional Cloning of the Ovine Callipyge Gene

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Grant 98-35205-6638; $160,000; 2 Years

Callipyge is a major gene responsible for pronounced muscle hypertrophy in sheep. The gene is associated with an increase (30%) in lean meat production and an 8% decrease in carcass fat in the lamb carcass. Genetic characterization of the locus has demonstrated a unique model of inheritance termed “polar overdominance", where only heterozygous offspring inheriting the mutation from their sire express the callipyge trait. The gene has been mapped to the distal end of ovine chromosome 18. The goal of this project is to develop a physical contig of ovine chromosome 18 that contains the callipyge gene. In addition, we will identify candidate genes within the region, orientating them within the contig. In this way, we will contribute to the identification of the causative gene for callipyge, leading to many exiting areas of study. Elucidation of the gene will allow better understanding of the relationship between muscle development, fat accumulation and tenderness. Possible manipulations of the gene may lead to improvement of carcass composition in other livestock species. Finally, characterization of polar overdominance may apply to other complex traits, including inherited diseases in man.

9803439 The Molecular Basis and Regulation of Colostrum Formation in Cattle

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Grant 98-35205-6708; $140,000; 2 Years

Colostrum, the first milk produced by cows after calving, provides essential antibodies from the mother that aid the calf in fighting disease-causing bacteria and viruses in the first few weeks of life. Colostrum is formed when the blood antibodies of the cow are transferred into the mammary gland in the last week of pregnancy. Our long term goal is to improve colostrum quality, increasing the antibodies available to calves to improve the resistance of calves to infectious diseases, as well as developing the ability to use antibody-enriched milk to increase the resistance of the cow to mastitis, and to treat infectious diseases in cattle, in other animals, and in humans while avoiding the use of antibiotics. The purpose of this project is to identify the molecular mechanism of colostrum formation. We have identified a candidate gene, similar to antibody transfer genes previously identified in mice and humans. In this project, we will first determine if the protein coded by this gene is the agent of antibody transfer into colostrum, and we will then try to determine how the expression of this gene is controlled by the mammary gland cell. This will lay the groundwork for developing the ability of cattle to produce improved colostrum to better protect young calves from pneumonia and diarrhea, and to produce antibody enriched milk at times other than the last week of pregnancy.

9803726 Mapping the Rainbow Trout Genome

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Grant 98-35205-6874; $196,142; 2 Years

Aquaculture of salmonid fishes is predicted to grow substantially in the near future. As cultivation of these species expands, it is important to determine the genetic basis of aquaculturally important traits such as growth rate, time of maturation, and disease resistance. This will allow fish breeders to select for fish with rapid growth rate, resistance to specific diseases and other traits which are important to aquaculture. In order to isolate genes for important phenotypic traits, it is necessary to have an integrated genetic map. The map needs to contain highly variable marker genes (such as microsatellite loci) which can be scored in virtually any cross. It is also important to map the previously cloned genes important in reproduction and physiology which might be candidate genes for phenotypic traits and to identify conserved blocks of genes which are present in other vertebrates such as the human and mouse with more detailed genetic maps. The major objectives of the proposed research are to prepare a comprehensive linkage map for rainbow trout using microsatellite loci and selected previously cloned genes. By tracing these genetic markers in the standard rainbow trout cross prepared by G. Thorgaard of Washington State University in which a variety of other genetic markers are being scored, we will be able to combine our results with those of the other laboratories in the US and around the world. The result will be a comprehensive linkage map for rainbow trout. Because of the conservation of gene order found in vertebrates, the rainbow trout map will also be useful to workers studying other aquaculture species such as catfish and tilapia.

9803433 Refined Mapping of Quantitative Trait Loci for Ovulation Rate in Cattle

Kirkpatrick, B.
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Grant 98-35205-6637; $180,000; 2 Years

Twin birth is commonly associated with diminished calf survival and poor cow reproductive performance. Consequently, it is a trait which is not desired by the majority of cattle producers. Twin birth in dairy cattle occurs on the order of 4-5% of the time. With lost revenue for a twin-bearing dairy cow estimated at more than $100 per head, twin birth causes an annual loss of revenue in excess of $35 million on a national basis. Nevertheless, under a suitable management program and sufficiently high twinning rate, it is possible to envision using twin birth in a more efficient beef production system. Successful mapping of genes for prolificacy will enable cattle breeders to produce animals through DNA testing which better fit their chosen management system, be it one favoring single birth or one favoring multiple birth. The primary objective of this research proposal is to more precisely identify the location of genes affecting frequency of twin birth. The proposed research builds from results of previous USDA grant-supported work which led to identification of four potential genes for twinning rate. These genes were identified within a Swedish-Friesian sire who was used extensively within a USDA cattle population intensely selected for twinning rate. Data and DNA from this population were used in the previous analysis. We propose again to leverage USDA's past investment in the twinning herd by using a closely related family from the same USDA herd to replicate gene effects and refine estimates of gene location.

ANIMAL HEALTH AND WELL-BEING

Panel Managers - Dr. Carole Bolin, USDA Agricultural Research Service, Dr. Mo Salmon, Colorado State University, Dr. Mark S. Kuhlenschmidt, University of Illinois
Program Directors - Dr. Peter J. Johnson, Dr. Peter R. Brayton

The objectives of this program are to increase the knowledge needed to sustain animal health and well-being and to prevent or reduce the severity of animal disease. This includes, but is not limited to: mechanisms that alter the normal physiologic state at the molecular, cellular or organ level to produce disease resulting from either biotic or abiotic causes; cellular mechanisms of disease resistance, including developmental and molecular immunology; microbial genetics/ genomics; pathogenesis; both host and microbial factors influencing colonization of mucosal surfaces; host-environment or host-agent interactions that compromise host defense systems or cause predisposition to disease; epidemiologic studies on animal diseases that provide insight into etiologic factors and/or disease control; research that supports the development or evaluation of diagnostic tests and immunizations for emerging or reemerging disease problems such as tuberculosis; studies on economic models that address the costs of animal disease and the cost/benefit ratios of animal disease prevention and therapy. The program also encourages research on the mechanisms controlling animal responses to physical and biological stresses (including quantitative behavioral, physiological, immunological and neurobiological responses to stress) and the development of objective indicators to measure animal well-being.

9802523 Test Dependence Affects Diagnosis and Surveillance of Animal Disease
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Grant 98-35204-6535; $149,906; 3 Years

Millions of dollars are spent annually in the United States to diagnose and monitor animal diseases. Often multiple tests are used for the same disease yet there is little evidence that effects of correlation/dependence between tests are considered in determining a cost-effective diagnostic strategy. In many cases, the number of tests might be reduced or diagnostic strategy changed to reduce the costs of testing to livestock producers without compromising animal health or trade. The overall goal of our research is to develop new methodology to assess performance of diagnostic tests and ultimately make diagnosis of animal disease and disease surveillance programs more efficient. Our first objective is to determine the effects of test dependence when multiple tests are used for diagnosis. To achieve this objective, we will use data on tests for bovine paratuberculosis, swine brucellosis, swine toxoplasmosis and beta-lactam antibiotic residues in cow milk. We will determine the optimal combination of tests to determine an individual's and herd's paratuberculosis status, incorporating a decision analysis approach. Determination of performance of multiple combined tests usually requires a definitive or absolute indicator of infection which is not always available for animal disease. To address this problem, we will use new statistical approaches to assess the validity of new tests in the absence of a standard test. Our second objective is to estimate the sensitivity and specificity of two or more tests in combination when there are more than two populations and no definitive test.

9802517 Epidemiology of Bovine Viral Diarrhea Virus Infection in Dairy Cows
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Grant 98-35204-6390; $150,000; 2 Years

Bovine viral diarrhea virus (BVDV) has caused devastating disease in Canada and the U.S., and infection without obvious signs is widespread among vaccinated herds. Information has not been available on the impact of chronic, 'silent' BVDV infection in cattle on typical commercial dairies. The aim of the research is to determine how BVDV impacts herd health and productivity, as well as why and when cattle become infected. We will develop a least-cost method to diagnose BVDV infection and field-based analytic and epidemiologic methods to determine a) how BVDV affects herd health and productivity and b) what management practices could be changed to reduce the impact of BVDV. The new diagnostic approach will permit cost-effective identification of BVDV-infected cattle, particularly those persistently infected (PI) that continually shed BVDV and infect other cattle. Epidemiologic methods will identify management practices and conditions that favor or retard BVDV transmission, including vaccination practices and exposure of calves to adults or to cattle with PI. These methods also will determine how BVDV affects dairy herd health and productivity, including morbidity, mortality, culling, and reproduction, particularly related to days open, heat cycles, services per conception, early embryonic death, and abortion. Estimates of the impact of BVDV on herd productivity will help determine costs of BVDV and the extent to which control efforts can be expected to minimize the economic impact of the disease for a dairy and for the industry.

9802413 Behavioral Activity in Broiler Chickens and its Effects on the Incidence of Skeletal Problems

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Grant 98-35204-6586; $170,000; 2 Years

One of the most important welfare problems affecting broiler chickens is the leg disorders associated with selection for rapid growth. These can impair mobility, making it difficult for the birds to walk to the feeder and the waterer, are probably painful, and cause economic losses to the industry due to the necessity to cull birds and the impairment of carcass quality. Despite selection by breeders for skeletal strength, 90% of commercial broilers still have detectable gait abnormalities. Modified lighting programs and feed restriction can be used to decrease leg problems, but each method has drawbacks. Another method to reduce leg problems might be to increase broiler activity. Increased activity has been linked to a decreased incidence of leg problems and an increase in blood flow to the legs. Broiler activity decreases with age, but the reason for this is unclear. The objectives of this study are to: 1) determine the relationships between specific leg problems and the degree of gait impairment; 2) evaluate activity patterns in broilers as a function of age, strain, behavioral profile, and gait impairment; and 3) develop behavioral methods to increase activity levels, for example by incorporating ramps and/or increased distances between the feeder and waterer; alternating feeding locations; and/or providing perches. Behavioral methods to decrease leg problems would improve the sustainability of US agriculture by providing a practical and inexpensive means to improve broiler welfare, production efficiency, and product quality.

9802472 Immuno-Pathogenesis of Bovine Trichomoniasis

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Grant 98-35204-6401; $130,000; 2 Years

Bovine trichomoniasis is a venereal disease of cattle that causes almost no sickness in adults, but results in enormous economic damage because it induces high rates of pregnancy losses. This project will test the general hypothesis that these pregnancy losses are the result of interaction between the immune response of the pregnant dam and the agent, *Tritrichomonas foetus*. Specifically, we will use cell culture methods to expose the putative "target" cells of this disease (*i.e.*, maternal uterine cells and fetal placental cells) to *T. foetus*, in the presence or absence of bovine antibody that is specific for the agent. In preliminary studies using white blood cells as model targets, we have shown that the killing power of the organism is greatly enhanced in the presence of systemic (IgG) antibody. That antibody reacts with a surface molecule that is shed by the organism in culture and adheres to the target cell. These studies will attempt to identify that shed molecule. They will also attempt to show that the same increased killing phenomenon occurs when the true target cells (uterine and placental cells) are employed; further, they will examine the protective role of an antibody type (IgA) that is secreted at surfaces, such as the cavity of the uterus; this antibody type may block the systemic antibody's harmful action. If the latter can be shown, it would suggest that vaccination strategies that enhance the surface (IgA)-type response should be more protective, and should be pursued.

9802336 Influence of RSV Infection on Immune Responses to Inhaled Antigens in Bovine Lung

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Grant 98-35204-6379; $180,000; 2 Years

Respiratory virus infection has often been associated with increases in allergy and asthma in humans. Some experiments using mice have shown that when a mouse is infected with a respiratory virus and breathes in foreign substances such as pollens, fungus, or dust it becomes allergic to those foreign substances more easily than if it breathed the same pollens, fungus, or dust without having a lung infection. We think that cattle may have the same type of response to foreign inhaled substances. Respiratory disease is extremely important to the cattle industry, causing great financial loss annually due to morbidity and mortality. Cattle are continuously exposed to dust, pollen, fungi, hay mites and other foreign substances that can cause allergy. In this project we will expose calves to respiratory syncytial virus and an aerosol of foreign protein and will test to see how their lungs and immune systems respond compared with calves that receive the same aerosol without the virus infection. We will use some new techniques, such as a lymphatic cannula, to study the cells and the molecules that are made in response to the inhalants and virus. The lung function of the calves will be compared so that we can determine if there is an effect on their ability to breathe. This research will help the cattle industry by defining some of the ways in which cattle become predisposed to getting severe respiratory disease and by providing information that will allow veterinarians to design better new treatment and prophylactic schemes.

9802453 Role of Porcine Beta-Defensin-1 (PBD-1) in Mucosal Immunity

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New Investigator Award; Grant 98-35204-6594; $163,000; 2 Years

The long-term goal of this project is to determine the role of porcine beta-defensin-1 (PBD-1) in mucosal innate immunity. We have obtained the cDNA of PBD-1 from porcine tongue epithelium. We hypothesize that PBD-1 is a potent antimicrobial agent that plays

an important role in porcine mucosal innate immunity against bacterial infections. Our specific objectives are: 1) to produce recombinant PBD-1; 2) to identify and purify native PBD-1; 3) to measure the concentrations of PBD-1 in mucosal fluids, and to evaluate the antimicrobial potency and spectrum of PBD-1 to swine pathogens as well as to standard laboratory testing strains. The proposed studies will provide new insights into the mucosal immunity against bacterial infections in pigs, especially in young pigs which have low or deficient immunity. The results of this work can benefit swine producers by creating new strategies for prevention and treatment of infectious diseases, such as deploying or inducing natural antibiotics where and when needed. The use of such natural approaches will also help inspire more public confidence in food quality by providing wholesome and safe food products.

9802215 Functional Analysis of *Haemophilus Somnus* Immunoglobulin Binding Proteins

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Grant 98-35204-6733; $170,000; 2 Years

Haemophilus somnus is a bacterial pathogen causing much economic loss in dairy and beef cattle. It is a major factor in the bovine respiratory disease complex, myocarditis, arthritis, reproductive failure and thrombotic meningoencephalitis. Most of these clinical syndromes are the result of the bacteria circulating in the blood and damaging the vessels. The goal of this research is to determine the mechanisms by which this bacterium survives in the blood, attaches to the blood vessel wall, invades the cells lining the vessels and causes tissue damage. To investigate these mechanisms, we will determine how the organism resists killing by blood serum so that it can circulate and multiply to cause tissue damage. Then we will determine how the organism attaches to bovine vascular endothelial cells in tissue culture and the mechanisms of toxicity. All these studies will involve the surface fibrillar network which binds immunoglobulin nonspecifically to the bacterial surface. After cloning and sequencing the genes from *H. somnus* immunoglobulin binding proteins (IgBPs), we identified several short sequences homologous with motifs of other organisms or cells responsible for binding or toxicity. The roles of these IgBP motifs will be explored. The results of these studies should be instrumental in devising new ways to control bovine disease caused by *H. somnus*.

9802346 Genetic Immunization for Control of Vesicular Stomatitis in Cattle and Horses

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Grant 98-35204-6398; $140,000; 2 Years

Vesicular stomatitis is a viral disease of cattle and horses. In the United States, major outbreaks of this disease have occurred regularly throughout this century, at intervals of roughly every 5 to 10 years. Most recently, outbreaks have occurred in the western states in 1995, 1997 and 1998. Vesicular stomatitis does cause morbidity and financial loss, particularly in dairy cattle, but the most significant problem is that cases in cattle and swine are clinically indistinguishable from foot-and-mouth disease, and outbreaks lead to rapid imposition of sometimes devastating interstate and international quarantines. Furthermore, animal health authorities must commit substantial resources to confirming that individual cases are vesicular stomatitis rather than foot-and-mouth disease. The goal of the proposed research is to develop an effective vaccine to protect cattle and horses against vesicular stomatitis virus. A critical constraint on such a vaccine is that it must allow easy differentiation of vaccinated from previously infected animals. Our approach is to develop a DNA vaccine in which animals receive an intradermal injection not of the virus or viral proteins, but of a gene that drives short-term expression of a viral protein in the animal. We will evaluate immune responses and protection from virus challenge in mice given one of several types of DNA vaccine, then test the best of those in cattle and horses. These studies are designed to not only contribute toward control of vesicular stomatitis, but to provide basic information on the use of this new vaccine technology in domestic animals.

9802281 Recombinant Chicken Interferons as Antiviral Agents

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Grant 98-35204-6954; $180,000; 2 Years

Poultry is the most widely used source of animal protein in the world, with an annual global consumption of about 19 billion chickens and a market value of about $60 billion. The poultry industry maintains extensive vaccine programs, along with biosecurity, to protect flocks against viruses that cause catastrophic economic losses due to compromised bird and egg production, and that in the extreme are lethal. These measures are not always adequate, as witness the depopulation of 18 million chickens in a 1984 influenza outbreak in the U.S., and over 14 million chickens in Mexico three years ago. The recent deaths of several people in Hong Kong resulting from an avian virus-derived influenza points out the need to control this virus. Vaccines are not always effective and may be circumvented by the ability of viruses to mutate into strains that do not respond to the original vaccine. A new approach to virus containment in the chicken industry is needed. To this end, we propose using a natural broad spectrum antiviral agent, interferon (IFN). Our laboratory has recently cloned the first chicken IFN gene, and with biotechnological procedures, can now produce enough IFN to test its efficacy in cells, eggs, and chickens. IFN acts within minutes to protect cells against virus infection, and hence might also serve to augment the action of slower acting vaccines. We propose to determine the spectrum of avian viruses that are sensitive to the antiviral action of chicken IFN, and define conditions that will deliver chicken IFN as an economically feasible antiviral agent that will improve and sustain U.S. agriculture.

9802057 2nd International Workshop on Molecular Pathogenesis of Marek's Disease Virus

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Grant 98-35204-6399; $5,000; 1 Year

This workshop will be held August 8-11, 1998 at Smolenice Castle, Bratislava, Slovakia. This workshop is a sequel to the First International Workshop on Current Developments in the Molecular Biology of Marek's Disease Virus (MDV) organized by the late Dr. Meihan Nonoyama and held in Tampa Bay, Florida in January of 1995. These workshops are intimate gatherings of 50-75 individuals representing leading laboratories worldwide involved in research on the molecular biology of MDV. They are held at four-year intervals interspersed between the large International Marek's Disease Virus Symposia. The workshops allow members of the international MDV research community, particularly those involved in molecular biology-related research, to meet and exchange information, ideas and insights every two years. Slovakia was chosen as the venue for this workshop because since 1988, both of the MDV meetings were held in the United States and the next International Symposium will be held in Montreal in 2000. Therefore, the International Steering Committee chose Slovakia as the location for the 1998 workshop. In addition, the poultry export market to Russia is one of the largest areas of growth potential for the U.S. poultry industry. It would greatly benefit U.S. scientists to visit former Soviet block countries and learn more about their industry and its market potential.

9802519 Society for Tropical Veterinary Medicine Biennial Conference; Key West, Florida, June 1999

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Grant 98-35204-6442; $8,700

The 1999 Conference of the Society for Tropical Veterinary Medicine (STVM) will be held in Key West, Florida. The theme of the conference is Tropical diseases; research on their control and prevention within the context of the "new world order". A "new world order" is emerging as we approach the next century. Within that context, research on the prevention, control, and eradication of tropical diseases is as much a reflection of economics, politics and societal wishes as it is the product of science and technology. The 3 day conference program will explore the opportunities and constraints of the "new world order" by holding symposia in the morning with "break out" sessions by discipline in the afternoons. An international perspective with speakers from around the world will be presented in the symposia; the focus will be on epidemiology, global trade, vaccines and diagnostic technologies. There will also be one afternoon field visit to federal and State organizations in Key West, charged with protecting the USA from the introduction of animal pathogens. The proceedings will be published in the Annals of the New York Academy of Sciences.

9802522 Opossum-Shed Sporocysts Capable of Inducing EPM

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Grant 98-35204-6487; $180,000; 2 Years

Equine protozoal myeloencephalitis (EPM) is a crippling neurological disease of horses in the Americas that is caused by a protozoan parasite named *Sarcocystis neurona*. Half of the horses in the US have been exposed to this parasite. In a percentage of the infected horses, the parasite replicates in the brain or spinal cord causing extensive nerve damage. This disease is considered by the US equine industry to be a serious threat to equine health. The form of *S. neurona* that is infective for the horse appears to be shed by the opossum. We have evidence that several other closely related species of Sarcocystis parasite also infect the opossum. The goal of our research is to obtain one or more *S. neurona* isolates from feral opossums, propagate the isolate through its complete life cycle in the laboratory, and demonstrate that it is capable of causing EPM in horses. Achieving this goal will allow us to study the disease in experimentally infected horses. In this project we will pursue these goals by completing the following two specific aims: 1) Survey free-ranging Florida opossums for *Sarcocystis* spp. infections, recover primary isolates as sporocysts from the intestinal tract and establish the isolates in cultures and infect suitable intermediate hosts; and 2) Compare primary parasite isolates with reference strains of *S. neurona* and *S. falcatula* by measuring morphologic, molecular, immunologic, and biologic characters, which may discriminate each as neurona-like, falcatula-like or dissimilar to either.

9802450 DNA Vaccines Against Anaplasmosis

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Grant 98-35204-6378; $150,000; 2 Years

Anaplasma marginale is a tick-transmitted rickettsial parasite of bovine erythrocytes that causes significant economic losses to the U.S. livestock industry. Current vaccines, although capable of conferring partial protection, are expensive to produce and have drawbacks that have stimulated the search for improved culture-derived or non-organism based vaccines. This has resulted in the *in vitro* culture of *A. marginale* and the characterization of major polypeptide constituents of outer membranes. A fundamental gap in our knowledge is definition of the most effective way to present *A. marginale* antigens, recombinant or cultured, to the bovine immune system to achieve protective responses. There is an increasing amount of evidence that cytokines produced early in the immune response determine the course of the T cell response, the cytokine pathways which develop, and in some cases, recovery from disease. Accumulating evidence

suggests that an immune response biased toward production of type 1 cytokines is necessary for protection against rickettsial infections, including *A. marginale*. DNA vaccines can induce type 1-biased, protective responses against infections, including against an infection caused by the related rickettsial agent *Cowdria ruminantium* in mice. We propose to test the hypothesis that: DNA vaccines against the MSP4 surface protein can induce a protective type 1 immune response in cattle against *A. marginale*.

9802614 Etiology, Diagnosis, and Prevalence of a Fatal Infectious Disease of Alligators

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Grant 98-35204-6731; $110,000; 2 Years

Commercial ranching of American alligators is an alternative commodity industry in the U.S. Gulf Coast states. Louisiana and Florida ranches produce about 150,000 hides and 340,000 kg of meat worth approximately $150 million annually. Because the industry is driven by collection of eggs and hatchlings from the wild, deriving economic value from alligators also encourages protection of wetlands necessary for their survival. Further, about 500,000 exported American alligators are held in 600 ranches in 47 countries for commercial production of hides and meat. A disease syndrome characterized by rapidly progressive paralysis, pneumonia and death emerged in captive alligators in Florida in 1995. Despite antibiotic therapy, water chlorination, and reduced population density, morbidity and mortality rates exceeded 85%. A new *Mycoplasma* sp. bacteria isolated from the blood, limb joints, internal organs, and central nervous system of affected alligators was implicated as the etiologic agent of the fatal disease. The pathogen may emerge from its reservoirs under conditions of captivity. This study will attempt to reproduce the natural disease by experimental inoculation of healthy alligators with a pure culture of the suspected mycoplasma, allowing the onset and progression of disease and development of alligator immune responses to be followed precisely. Blood samples collected after the experimental inoculations will serve as standards necessary to validate an immunological diagnostic test for exposure to the pathogen. The test will have direct application to ensure that alligators used or transported for commercial purposes, or returned to the wild, are free of this infectious disease.

9802206 Molecular Approaches Toward the Control of Ichthyophthirius Infection

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Grant 98-35204-6812; $300,000; 3 Years

Losses due to parasitic disease cost fish farmers an estimated $25 million a year. The protozoan, *Ichthyophthirius multifiliis* (commonly referred to as *Ich*), is one of the most serious pathogens of freshwater cultured fishes and its impact increases as the aquaculture industry grows in the U.S. The goal of our research is to develop a vaccine against this important parasite. We use channel catfish in our studies because they are readily available, easily maintained, and highly susceptible to infection. In addition, channel catfish farming is one of the most rapidly expanding areas of agribusiness and represents the largest segment of U.S. aquaculture. A vaccine against *Ich* will reduce the incidence of disease, increase production efficiency, and eliminate the use of chemicals that contaminate surface and groundwater with hazardous residues. Our work will also contribute to a basic understanding of the immune response of fishes to microbial pathogens. Because *Ich* cannot be grown in culture, we are introducing selected genes into the non-pathogenic, free-living ciliate *Tetrahymena thermophila* to produce protective *Ich* antigens in this easily cultured organism. Expression of native *Ichthyophthirius* proteins on the surface of *Tetrahymena* may provide the most efficient means of exposing fishes to protective antigens, short of infection with the parasite itself. Furthermore, the *Tetrahymena* expression system should be of widespread use in the development of other fish vaccines.

9802627 Catfish Nonspecific Cytotoxic Cells: Receptors and Signaling

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Grant 98-35204-6701; $150,000; 2 Years

Nonspecific cytotoxic cells (NCC) are the best characterized effectors of innate cytotoxic immunity in catfish. NCC are the teleost equivalent of mammalian natural killer (NK) cells and as such provide the first line of defense against infections by protozoan parasites, bacteria, viruses and growth of tumors. Target cell recognition and conjugate formation by teleost NCC is accomplished by a membrane receptor protein (NCCRP-1). NCCRP-1 is also found on equivalent (NK) cells from many mammals including man, rats and numerous other animal species (e.g. bovine, canine, equine, cervine, chickens, etc.). We have sequenced the complete NCCRP-1 membrane receptor protein. NCCRP-1 can be divided into three functional segments. One portion contains signature amino acid sequences that are also characteristic of human cytokine receptor and growth hormone receptor sequences. These unique amino acids (or motifs) provide docking sites for cytoplasmic kinases. This interaction transduces signals or messages to the NCC nucleus and activate certain cytokine genes. The cytokines probably function to amplify inflammatory responses. Other portions of the NCCRP- I receptor molecule come in direct physical contact with a target antigen on protozoans or tumor cells. This binding is responsible for initiation of the NCC lytic cycle. Our research suggests that NCCRP- I may orchestrate all NCC functions in innate immunity. The specific goals of this proposal are: to identify the molecular processes of NCCRPI gene regulation and characterize the cytoplasmic enzymes that are responsible for NCCRP- 1 gene transcriptional activation. These studies will determine the genomic organization of the NCCRP- I gene(s). This information is essential for understanding transcriptional processing and membrane expression of NCCRP-1. The existence of the highly

conserved signature motifs suggest that NCCRP-I may belong to a gene family of conserved proteins important in immunoregulation and lymphocyte growth.

9802284 The Role of *Rev* in Lentivirus Persistence

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Grant 98-35204-3847; $198,000; 2 Years

Lentivirus infections of domestic animals are characterized by a high rate of genetic variation and prolonged periods of virus persistence. The goal of this project is to better understand the relationship between genetic variation and virus persistence. Some viruses persist by shutting down virus replication, allowing the virus to hide from the immune system. The lentivirus protein *rev* controls levels of virus replication and is required for production of infectious virus. We found a high rate of genetic variation in a regulatory gene, *rev*, of equine infectious anemia virus (EIAV). We demonstrated that sequence variation in *rev* changes levels of virus replication. *In vivo*, this may allow the virus to hide from the immune system. In this proposal, we will determine if variation in *rev* plays a role in EIAV persistence. In the first objective, we will isolate virus from sequential sera samples taken from ElAV-infected horses at various stages of clinical disease and determine the extent and location of sequence variation in the *rev* gene. In the second objective, the *rev* proteins will be functionally tested to determine the effect of variation on *rev* activity and virus replication. Comparison of activity among *rev* variants isolated during clinical and aclinical periods of disease will be used to determine if *rev* plays a role in lentivirus persistence. Lentivirus infections occur naturally in a number of domestic animal species. Understanding the means by which these viruses persist in infected host animals will help to identify potential targets for vaccine or drug therapy.

9802539 Potentiation of PRRSV-induced Pneumonia by Mycoplasma: Role of Pro-Inflammatory Cytokines

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Grant 98-35204-6441; $270,724; 3 Years

Porcine respiratory disease complex (PRDC) is an economically significant respiratory disorder in growing pigs estimated to affect 10 million animals annually with an economic loss to the swine industry in excess of $40 million since 1993. Diagnostic laboratories have found that porcine reproductive and respiratory syndrome virus (PRRSV) and *Mycoplasma hyopneumoniae* (*M. hyo*) are most frequently isolated from grow-finish pigs with clinical signs of PRDC. A recent study completed in our laboratory demonstrated that infection with *M. hyo* significantly increased both the severity and the duration of PRRSV-induced pneumonia. The objective of this proposal is to begin investigating possible mechanisms to explain how *M. hyo* increases the severity of PRRSV pneumonia. We propose that inflammation induced by *M. hyo* results in increased tissue destruction in the lungs which further attracts PRRSV susceptible pulmonary alveolar macrophages. The proposed study will investigate the role that proinflammatory cytokines play in increasing inflammation leading to pneumonia. Both *in vivo* and *in vitro* systems will be used to evaluate the interplay between these two pathogens. Two specific aims will be pursued: (i) establish the levels of proinflammatory cytokines including tumor necrosis factor alpha (TNF), interleukin-1 (IL-1) and interleukin-6 (IL-6) and PRRSV concentrations in pigs infected with *M. hyo* and/or PRRSV; and (ii) evaluate the effect of *M. hyo*-infected epithelial cells on PRRSV infected macrophages in vitro. Understanding the pathogenesis involved in the interaction between PRRSV and *M. hyo* will aid in the development of improved intervention and therapeutic strategies for controlling PRDC.

9802514 Treatment of Porcine Diarrhea by Epithelial Apical Anion Conductances

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New Investigator Award; Grant 98-35204-6400; $130,000; 2 Years

Secretory diarrhea ('scours') accounts for at least $70 million in losses to the swine industry in the United States annually. However, proteins that transport salt across the cells which lead to the watery secretion have not been pharmacologically identified in either the small intestine or the colon. Thus, drugs which selectively block these proteins have not been described. Compounds which directly block the appropriate proteins would be particularly useful for acute symptomatic treatment in veterinary practice because of their activity against a broad spectrum of pathogens without the caveat of selection for antibiotic resistant bacterial strains. We have identified a family of compounds, diarylsulfonylureas, that selectively modulate the activity of the apical channel responsible for intestinal chloride secretion in humans, the cystic fibrosis transmembrane conductance regulator (CFTR). Preliminary data shows that these compounds inhibit chloride secretion that has been stimulated by a variety of neurotransmitters and second messengers in cultured human colonic epithelial cells and in rat colon. These compounds, likewise, reduce electrolyte secretion in pig colon. We propose to employ diarylsulfonylureas and blockers of other known Cl- channels to identify the portion of stimulated ion transport mediated by each conductance in pig intestine, to directly verify the mechanism of action of diarylsulfonylureas on the porcine homologue of CFTR, and to further optimize this family of compounds for inhibition of toxin-induced intestinal secretion. The primary goals of this project are to identify both the conductance(s) responsible for pathological salt secretion and a drug which potently and selectively inhibits this conductance.

9802531 Porcine Antimicrobial Peptides

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Grant 98-35204-6397; $240,000; 3 Years

The long-term goal of this project is to devise strategies to control the expression of porcine antimicrobial peptides at certain times and in specific cells that will limit pre-harvest spread of important porcine and human pathogens. Our studies on the cathelicidin antimicrobial family coupled with our recent discovery of a porcine epithelial antimicrobial peptide, porcine β-defensin- 1, provides a timely opportunity to use this information to determine if natural antimicrobial peptides can be used to devise effective disease defense strategies for young pigs. Our interest in natural antimicrobial peptides stems from our desire to find alternate means of addressing the issue of mass medication in food animals, particularly in view of the intense scrutiny and criticism that antibiotic usage in food animals continues to receive. Understanding and devising strategies to modulate natural antimicrobial peptide capabilities may decrease the use of antimicrobial agents. Using less antibiotics in food animals will increase the consumer's confidence in a wholesome quality pork product, yet alternate antimicrobial strategies such as enhancing natural antimicrobial peptide capabilities, will allow pork producers to maintain efficiency in their swine units, which will contribute to the sustainability of U.S. agriculture.

9802270 Identification of *Edwardsiella ictaluri* Virulence Factors

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Grant 98-35204-6703; $180,000; 3 Years

Commercial catfish production accounts for 85-90% of the total fin fish aquaculture production in the United States, with almost 500 million pounds, valued at $2.5 billion dollars, produced in 1996. Enteric Septicemia of Catfish (ESC), caused by *Edwardsiella ictaluri* and recognized as the number one disease problem facing the catfish industry, was recently reported to be present on 56% of catfish operations in the U. S., and on 83.7% of operations with 150 acres or more of production. Measured only in direct loss of fish, ESC mortalities result in losses of about 25 million pounds, or roughly $ 19 million. Control of ESC is currently limited to the only two antibiotics cleared for use in catfish, Terramycin and Romet, and an increasing number of isolates are resistant to both drugs. Killed vaccines demonstrate limited success, and the only licensed product is unavailable because of high cost and low efficacy.

This project is based on the hypothesis that basic knowledge of ESC pathogenesis and virulence factors is important to the ultimate control of disease. Consequently, the long term goal of this project is to identify and characterize *E. ictaluri* virulence factors using signature-tagged mutagenesis, a newly developed procedure that allows the simultaneous production, screening, and characterization of a large number of isogenic mutants. Evaluation of those mutants will lead to an expanded knowledge of *E. ictaluri* pathogenesis and virulence factors, and will provide insight that can subsequently be applied to new drug and vaccine strategies for the prevention and control of ESC.

9802072 Molecular basis of stress-induced immune modulation

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Grant 98-35204-6488; $180,000; 2 Years

The contribution of stress towards economically important diseases of animals has been widely recognized for a number of decades. Numerous studies have been able to link specific stress-induced factors with changes in immune function. Nevertheless, questions remain regarding the precise mechanism of stress-induced immune modulation. The focus of the studies described in this proposal are to define the mechanism of stress-induced changes in immune responses. Our overall approach will be to subject a group of horses to a defined stressor (exercise) and to characterize the mechanism of stress-induced alterations in the ability of their lymphocytes to proliferate in response to various stimuli. The proliferative response of lymphocytes involves several key processes. We propose to focus on the initial events in lymphocyte activation and proliferation by: (1) Characterizing the effect of exercise stress on the early events in lymphocyte activation. These studies will look at some of the earliest intracellular events that occur following the stimulation of the lymphocytes and how stress may alter these responses. (2) Determining the effect of exercise stress on signal transduction via the interleukin2 (IL-2) receptor. Lymphocyte proliferation is dependent upon the growth factor IL-2 and its interaction with its receptor on the lymphocyte cell surface. These studies will focus on how stress might alter the intracellular signaling pathway involving IL-2 and its receptor. An improved understanding of the molecular basis of stress-induced immune function will lead not only to a better understanding of immune regulation but may also identify potential targets for therapeutic intervention.

9802391 Genetic Characterization of Emerging Bovine Respiratory Coronaviruses Kousoulas, K**.** G.; Storz, J.

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Grant 98-35204-6585; $182,000; 3 Years

Bovine coronaviruses are associated with enteric disease in adult cattle. Recently, we discovered a strong association of respiratory bovine coronavirus infections with acute respiratory disease in cattle including “shipping fever” and acute pneumonia. Preliminary

characterization of a small number of viruses revealed that all respiratory viruses were closely related and contained specific genetic alterations in comparison to enteric viruses. Respiratory viruses exhibited different properties in cell culture in comparison to enteric strains including differential ability to aggregate chicken and rabbit red blood cells and increased amount of cell fusion. The proposed work aims to characterize genetically numerous respiratory bovine coronaviruses isolated from infected cattle and to compare their genetic and functional properties to bovine coronaviruses which were isolated from the enteric tracts of other cattle. Our overall hypothesis is that all respiratory bovine coronaviruses are closely related, and that their unique properties in cell culture are principally due to specific amino acid and nucleotide changes. Therefore, we intend: (1) To genetically characterize respiratory bovine coronavirus strains isolated from cattle with shipping fever and (2) To analyze and compare the structure and function of independently expressed S and HE glycoproteins specified by respiratory and enteric bovine coronaviruses. Expected benefits for the U.S. agriculture include: (i) Understanding of how these viruses rapidly emerged to become the predominant viral respiratory track pathogen of cattle, (ii) The development of sensitive molecular tools for detecting and differentiating respiratory from enteric coronaviruses, (iii) The design of vaccines for the effective prevention of all bovine coronavirus infections.

9800664 2nd International Conference - Novel Approaches for Controlling Livestock Helminths

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Grant 98-35204-5901; $9,000; 1 Year

Helminth parasites remain to be an important limiting factor in livestock health and production world wide. In light of the almost exclusive use of chemicals in the control of these agents, alternate strategies are necessary to reduce costs, produce residue free animal products, decrease potential environmental contamination, and control these agents as drug resistant parasite populations increase in prevalence. This award provides partial support for the 2nd international conference on Novel Approaches to the Control of Helminth Parasites of Livestock to be held at Louisiana State University in Baton Rouge, March 22 through March 26, 1998. In addition to registration fees other funding has been promised or is in place from a number of sources including Louisiana State University, the pharmaceutical industry, Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA, Brazil), and funds remaining from the 1st Novel Approaches conference held in Australia in 1995. The overall goal of this conference is the integration of novel parasite control methods into sustainable systems of livestock management. The specific objectives are to: 1. Examine the status of the approaches currently being studied by critical open debate; 2. Identify the information yet needed for the realistic implementation of these technologies in the field; 3. Foster an environment conducive to the establishment of working relationships between individuals in different disciplines; and 4. Disseminate information and discussion generated through publication in the International Journal for Parasitology. This will be accomplished through a workshop and poster format for the meeting.

9802620 Role of the Siderophore DHBA in the Virulence of *Brucella abortus* in Cattle

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Grant 98-35204-6704; $200,000; 2 Years

Brucella abortus has been reported to produce a single siderophore, 2,3-dihydroxybenzoic acid (DHBA), under conditions of iron starvation. Evaluation of an isogenic DHBA negative mutant (BHBl) constructed from virulent *B. abortus* 2308 indicates that the production of this siderophore is required for virulence in pregnant goats. Interestingly, DHBA production also appears to be required for optimal growth of *B. abortus* 2308 in the presence of erythritol under iron-limiting conditions in vitro. Massive replication of *B. abortus* within the erythritol-rich trophoblasts surrounding the developing fetus during the latter stages of pregnancy is believed to be a major determinant of fetal abortion, the predominant clinical presentation associated with bovine brucellosis. We propose that DHBA provides the brucellae with sufficient levels of Fe+++ to maintain the rapid and extensive growth which takes place within ruminant placental trophoblasts. In doing so, this siderophore serves as an important virulence factor for *B. abortus* in the gravid ruminant uterus. To test our hypothesis, we will evaluate the capacity of BHB1 to replicate and produce cytotoxicity in cultured bovine placental trophoblasts and examine the pathogenicity of this mutant in cattle. The genetic organization of the *B. abortus* DHBA biosynthesis operon, along with the physiologic basis for the DHBA requirement exhibited by *B. abortus* during erythritol metabolism will also be examined. Defining the bacterial components which allow the brucellae to sustain their prolific growth with placental trophoblasts will provide us with a better understanding of the pathogenesis of *B. abortus* in the ruminant reproductive tract. Such knowledge will provide the basis for designing improved vaccines and therapeutic approaches for bovine brucellosis.

9802269 Third International Symposium on Aquatic Animal Health

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Grant 98-35204-6474; $8,500; 1 Year

The Third International Symposium on Aquatic Animal Health, to be held August 30th through September 3rd, 1998, at the Renaissance Harborplace Hotel - Baltimore, Maryland, will unite the expertise of aquatic animal health researchers, practitioners and managers from around the world. The symposium will provide the first major international forum for presentation and exchange of

information on an important diversity of freshwater and marine species including crustaceans, molluscs, cephalopods, fish and marine mammals. The objectives of the research conference include (1) the exchange of up-to-date information on infectious and non infectious diseases of aquatic animals, including disease prevention and treatment, (2) fostering national and international cooperation to support improvements in the health of invertebrates and fish important for aquaculture, and (3) dissemination of current information on control and certification procedures, particularly regarding pathogens listed in the OIE (L'Office International des Epizooties) Aquatic Animal Health Code. The format for the symposium will include plenary lectures, oral and poster presentations, discussion groups and workshops, and visits to local aquatic animal health research facilities. The symposium will also host and integrate the meetings of the 1998 Fish Health Section of the American Fisheries Society, and the Aquaculture and Seafood Advisory Committee of the American Veterinary Medical Association. USDA funding, in part, will be used to produce the symposium plenary and contributed oral sessions. The subject matter focus of these sessions, and the symposium in general, is aquatic animal health and well-being. Additional symposium information can be obtained on the worldwide web (http//som 1.b.umd.edu/AquaticPath/isaahweb).

9802051 Interferon-gamma Mediated Enhancement of Protective Host Immunity to Coccidiosis
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Grant 98-35204-6471; $95,926; 2 Years

Coccidiosis is an ubiquitous intestinal protozoan infection of poultry that seriously impairs the growth and feed utilization of the infected animals. The ability to vaccinate for the control of coccidiosis would reduce the current annual loss of $600 million and would have a major impact on the poultry industry world-wide. However, an effective vaccine against coccidiosis does not currently exist. Understanding the mechanisms of intestinal cellular immunity that confer protection is crucial for the development of a coccidial vaccine. The long term goal of this project is to develop a novel control strategy for effective oral immunization of chickens in order to induce a protective mucosal immune response against *Eimeria*. Our hypothesis is that the *Eimeria*-induced local production of interferon-γ is critical for protection against coccidiosis. The immediate objectives of this project are to produce a biologically active recombinant chicken interferon-γ (rchIFN-γ) to test its adjuvant effect on vaccination of chickens with live parasites and recombinant *Eimeria* antigens. The successful accomplishment of this research project will provide a rational basis for using this cytokine as a vaccine adjuvant against coccidiosis and will allow the development of a practical and effective immunological control strategy for *Eimeria*.

9802290 Production of Infectious Newcastle Disease Virus from cDNA: Potential for Vaccine Development and Basic Studies
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Grant 98-35204-6427; $140,000; 2 Years

Newcastle disease is one of the most infectious diseases of poultry in the world. Some Newcastle disease virus (NDV) strains can cause 100% mortality in unvaccinated poultry flocks. NDV remains as a major threat to the U.S. poultry industry. Live attenuated vaccines are widely used to control Newcastle disease. The main disadvantage of live NDV vaccines is that they can cause disease and can lead to mortality. Thus, development of a completely apathogenic NDV vaccine would be beneficial to the poultry industry. At present, there is no method available to genetically engineer attenuated vaccine strains. Our long-range goal is to develop safe, effective and inexpensive live attenuated vaccines for Newcastle disease using genetic engineering techniques. These vaccines will be generated in the laboratory by directly introducing changes into the genetic material (nucleic acid) of the virus. As a first step toward this goal we propose to construct a full-length copy of the genetic material of NDV in our laboratory so that it can be manipulated in the future. The full-length copy of the BRSV genetic material will be used to produce infectious NDV in cell culture. In addition, the manipulation of the genetic material of NDV will be useful for basic studies of NDV molecular biology and pathogenesis.

9802348 Molecular and Biological Analysis of the Emerging Avian Retrovirus ALV-J
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Grant 98-35204-6393; $140,000; 2 Years

According to the National Agricultural Statistics Service of the USDA, 8.08 billion broiler-type chicks were hatched in the US in 1996. This industry is now threatened by the recent emergence of a new virus, designated ALV-J, that induces myeloid tumors in susceptible chickens. This virus appears to be a heterogeneous mixture consisting of distinctly different members. Our research goals are designed to identify the most representative ALV-J members among our numerous field isolates. These viruses will form the basis for future efforts to develop effective diagnostic tools and new vaccines that will be appropriate to detect and control the full spectrum of ALV-J isolates. We will also determine whether ALV-J induces myeloid tumors as a result of a gene carried in the virus or through activation of a chicken gene. This information will help in understanding how quickly ALV-J can mutate and whether we might expect new pathogenic variants to emerge.

9802202 *In vivo* expressed genes of *Actinobacillus pleuropneumoniae*
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Grant 98-35204-6702; $290,000; 3 Years

Porcine pleuropneumonia, caused by the bacterium *Actinobacillus pleuropneumoniae* (APP), is a severe and economically important contagious respiratory disease of swine. The ultimate goal of our research is to prevent APP infection. To achieve this goal we need to understand how APP causes infection in swine, and in particular to identify the factors that contribute to the virulence of APP and the antigens that trigger a protective immune response in swine. The objective of this research project is to identify and analyze APP genes that are turned on, or expressed, when the bacterium is within a pig (*in vivo*), but are not turned on during growth of the bacterium in the laboratory. The underlying hypothesis of this research is that *in vivo* expressed genes will encode virulence factors of APP that either are involved in the production of disease or are critical to survival of the bacterium in the host animal. We have developed a genetic system that allows identification of these *in vivo* expressed genes and have already identified six genes that fit these criteria. We will continue to identify additional genes, and analyze the role each plays in the ability of APP to cause disease. Identification of these genes and their products will lead to improved understanding of how APP, and other related bacterial respiratory pathogens, causes disease and to new avenues for the development of vaccines against APP and new targets for the development of antibiotics.

9802052 Development of Heat-Labile Enterotoxin (LT) as Carrier of Foreign Antigen Epitopes

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Grant 98-35204-6734; $156,812; 2 Years

We propose to construct recombinant vaccines against porcine transmissible gastroenteritis virus (TGEV) by inserting specific fragments of viral proteins into the A subunit of the heat-labile enterotoxin (LT) from *E. coli*. Fragments to be inserted will be selected such as to constitute the immunogenic domains of the viral proteins. They will be inserted as part of the LT by manipulation of the genes encoding the A and/or the B subunits. Briefly, synthetic oligonucleotides encoding appropriate fragments of the viral proteins will be inserted into the genes of A and/or B subunit, the recombinant proteins will be produced in bacteria, purified and used to vaccinate experimental animals, such as rabbits or mice. The antiserum obtained from these experimental immunizations will be tested for the ability to inactivate the virus. Recombinant proteins that will give the best virus inactivation results will be used to immunize pigs in the virus challenge experiments. Porcine gastroenteritis is an important infectious disease of agricultural food supply animals. It causes significant losses to agricultural production. No reliable vaccine is available at present.

9802342 Expression of Soluble Receptor and Envelope Proteins as an Antiviral Strategy

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Grant 98-35204-6392; $286,000; 3 Years

The ability to artificially introduce new genes into the germline of agriculturally important animals offers a new avenue to acquire genetic traits useful to improve the breeds. We have concentrated on developing useful strategies to increase disease resistance since this trait seems particularly hard to improve by conventional breeding schemes. We have focused on developing strategies that confer disease resistance against retroviruses as a model system with the long-term goal of extending any strategies that were successful to other viral pathogens that affect livestock. The antiviral strategy described in this proposal targets the initial step of the retroviral life cycle: the interaction of the virus with the host cell. Specifically, we hypothesize that retroviral infection of cells can be prevented by expressing soluble forms of the proteins involved in this initial interaction, the host receptor and viral envelope glycoproteins. We have set out to determine if the expression of soluble receptor and/or envelope proteins from a gene delivered by a retroviral vector can effectively interfere with a virus infection. This research will increase our understanding of the initial steps of retroviral infection, and explore potential strategies to disrupt disease progression. Two retroviruses that are significant pathogens of commercial poultry flocks, avian leukosis virus and reticuloendotheliosis virus, will be studied.

9802232 Whole-genome sequencing of *Pastuerella multocida*

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Grant 98-35204-6732; $200,000; 2 Years

The overall goal of this project is to determine the complete DNA sequence of *Pastuerella multocida*, a bacterium that causes fowl cholera in poultry, hemorrhagic septicemia in cattle, and atrophic rhinitis in pigs. Together, this bacterium is responsible for several hundred million dollars worth of annual economic loss to animal production. Despite the great economic impact of disease due to *P. multocida*, very little is known about how this organism causes disease. Hence, it is essential to learn as much as possible about the organism itself, including it's complete repertoire of genes and potential virulence determinants. We propose to initiate a program to determine the complete nucleotide sequence of the *P. multocida* genome by adopting a strategy that has previously been applied for sequencing the genomes of microbial pathogens. In brief, we will construct a random plasmid library from a common avian strain of *P. multocida*, and sequence the ends from 6000 plasmid clones. The subsequent sequencing of the ends of additional plasmid and bacteriophage clones will enable the determination of the entire sequence. Database similarity searches will be used to assign genes with

functional roles, and the sequences deposited in public genetic databases. This will enable researchers to access information on the sequences and other features associated with the genome of *P. multocida*. This study will facilitate future research on questions relating to bacterial virulence and pathogenicity and enable the development of new classes of antimicrobial agents and identify antigens that may be used as vaccines.

9802328 Mechanism of Pestivirus NS2-3 Processing and the Effect on Cytopathogenicity

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Postdoctoral Fellowship; Grant 98-35204-6440; $75,000, 2 Years

Bovine viral diarrhea virus (BVDV), is a clinically and economically significant bovine pathogen which can cross the placenta and infect the developing fetus. Depending on the age of the fetus: abortions, stillbirths, fetal malformations or the birth of apparently healthy offspring may result from *in utero* infections. Importantly, infection during the first trimester may result in persistently infected animals which shed virus for the rest of their lives. Sporadically, persistently infected cows develop fatal mucosal disease (MD). Interestingly, viruses obtained from persistently infected cows are noncytopathic (ncp) in cell culture. In contrast, cytopathic (cp) BVDV, and ncp BVDV are isolated from cows with MD. In all cases of cp BVDV, a viral protein, NS3, is produced in addition to the NS2-3 protein, whereas only NS2-3 is found in ncp BVDV. To determine the role of NS2-3 processing and the appearance of NS3 in the development of cytopathogenicity, I propose to use a viral protein expression system to map the 2/3 cleavage site in BVDV NADL and examine the effect of cleavage site mutations on NS2-3 processing. To examine the genetic determinants of cytopathogenicity, mutations which alter NS2-3 processing and the production of NS3 will be introduced into our cp and ncp infectious NADL cDNA clones. The levels of viral RNA replication, appearance of cytopathic effects, polyprotein synthesis and processing will be determined for the derived wild-type and mutant viruses. These studies may identify attenuated BVDV viruses which can be used as potential vaccines.

9802214 Analysis of the Neutrophil NADPH Oxidase in Bovine Mastitis

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Postdoctoral Fellowship; Grant 98-35204-6814; $90,000; 2 Years

Neutrophils play an essential role in the cellular defense of the bovine mammary gland, and compromised leukocyte function has been linked to the development of bovine mastitis, which is the most costly disease afflicting dairy cows worldwide. When an invading organism enters the mammary tissue in normal cows, large numbers of neutrophils migrate into this tissue and become activated, resulting in the assembly of neutrophil proteins to form a superoxide anion-generating complex known as the NADPH oxidase. The proposed studies focus on investigating two fundamental hypotheses: 1) that low molecular weight GTP-binding proteins function as molecular switches to regulate the assembly and activation of the bovine neutrophil NADPH oxidase and 2) that modulation of the bovine neutrophil oxidative burst is involved in the frequency and severity of bovine mastitis and possibly other bovine infections. To investigate these hypotheses, the following studies are proposed: 1) cloning and sequencing bovine low molecular weight GTP-binding proteins and expressing the cloned proteins, 2) analyzing the bovine NADPH oxidase proteins in milk neutrophils from healthy cows and cows with mastitis, and 3) analyzing how the bovine NADPH oxidase is regulated in neutrophils isolated from the milk of normal and diseased cows. Completion of these studies will increase our understanding of the role the neutrophil oxidative burst plays in bovine mastitis. The information gained may eventually lead to the development of therapies that could modulate the oxidative burst and enhance the bovine host defense processes.

9802473 Control of the Coccidian Replicative Cycle

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Postdoctoral Fellowship; Grant 98-35204-6391; $90,000; 2 Years

Toxoplasmosis is a serious disease in animals causing mortality in newborn pigs and abortions in sheep, as well as human birth defects and encephalitis in AIDS patients. Tissue cysts (bradyzoites), ingested in meat products, is one route of disease transmission. Evidence suggests a cell cycle mechanism may regulate tachyzoite-to-tissue cyst development in the intermediate host. Tachyzoites arising from a sporozoite infection exit early host cells replicating once each 6 h, but this rapid growth is restricted and following a spontaneous slowing, tachyzoites are observed to replicate once each 15 h. The shift to a slower cell cycle appears essential to the tachyzoite developmental program, since bradyzoite differentiation ensues only subsequent to slowed growth. Thus distinctly different cell cycles are potentially critical to the formation of the tissue cyst. We have a stable transformed tachyzoite strain containing the gene for Herpes simplex virus-2 thymidine kinase. Exogenous thymidine will reversibly arrest the growth of this parasite at the G1-S boundary of the cell cycle. Using this model, we will establish cell cycle differences between tachyzoite isolates that display fast verses slow growth phenotypes. Two approaches will also be employed to isolate molecules that may regulate tachyzoite growth and differentiation to the bradyzoite. We will generate a panel of mutant tachyzoites that are conditionally defective for cell cycle proteins allowing for the functional identification of essential molecules. In a parallel approach, we will examine gene expression differences in tachyzoites that switch from the fast to the slow cell cycle using differential mRNA display.

9802264 Role of *E. coli* Heat-Labile Enterotoxin in Diarrhea and Septicemia in Swine

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Grant 98-35204-6761; $140,000; 2 Years

Despite the fact that nearly half of all maternal swine are vaccinated for enterotoxigenic *Escherichia coli* (ETEC), this bacterium remains the most common cause of diarrhea in nursing pigs. Porcine ETEC strains are thought to cause diarrhea mainly by the production of enterotoxins, but this has not been definitively established. Strains that are particularly virulent also may infect the bloodstream and cause a highly lethal, toxic condition known as septicemia. The mechanisms by which this occurs are unknown. The central hypothesis of this research is that heat-labile enterotoxin-I (LT-I) is essential for the development of severe diarrhea but not septicemia in swine. This hypothesis will be tested by experiments involving an LT-I+ ETEC strain isolated from a pig with diarrhea and septicemia. A stable mutation in a gene in this strain will be introduced to make it unable to produce biologically active LT-I molecules (LT-I-). A complemented (LT-I+) clone of this mutant strain will then be constructed by introducing a copy of the intact gene into the mutant strain. The abilities of the parent (LT-I+), mutant (LT-I-), and complemented mutant (LT-I+) strains to cause diarrhea and septicemia in piglets that are germfree before inoculation will be compared. The results of this research should help in the formulation of new strategies to reduce illness and mortality due to ETEC infection. Through reduced illness and mortality in swine, this research should help to increase the productivity and competitiveness of U.S. agriculture and help to maintain an adequate food supply.

9802537 Mechanisms of Intestinal Repair in the Horse

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Grant 98-35204-6470; $150,000; 2 Years

The objective of this research program is to study mechanisms of intestinal repair in horses suffering from colic. Colic refers to any cause of abdominal pain, and kills more horses than any other disease syndrome. Colic results in annual monetary losses of approximately $250 million in a horse industry producing $25.3 billion annually. This grant, aimed at hastening recovery of horses with colic, represents the major thrust of the research mission of the Carolina Performance Horse Program. Our preliminary studies indicate that prostaglandins stimulate recovery of injured intestine, but the repair process is interrupted by an aggressive inflammatory process. Additional studies show that the nutrient genistein augments intestinal recovery in the presence of prostaglandins. We hypothesize that treatment with a combination of prostaglandins and genistein will overcome the inflammatory response and trigger early recovery of injured intestine. We will pre-treat animals with antibody which prevents infiltration of inflammatory cells to determine the role of inflammation. We will test if oxidants produced during active inflammation are responsible for disrupting repair by pre-treating with anti-oxidants. Finally, we will assess if genistein in combination with prostaglandins augments intestinal repair despite active inflammation. These studies will provide a greater understanding of the intestinal repair process, and allow development of novel treatments for horses with acute colic.

9802064 Analysis of Apoptosis and Pathogenesis by Bovine Herpesvirus 1 and bICP0.

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Grant 98-35204-6377; $178,338; 3 Years

Bovine Herpesvirus 1 (BHV-1) is a significant viral pathogen of cattle which can cause upper respiratory disease, abortions, "shipping fever", or occasionally encephalitis. BHV-1 establishes a latent infection in the peripheral nervous system, primarily sensory neurons located in trigeminal ganglia of an infected host. Virus can persist in a latent state for the lifetime of the infected host or it can periodically reactivate. The ability of the virus to establish a latent infection and reactivate from latency is the main reason why BHV-1 is maintained in the field. Our preliminary studies have demonstrated that programmed cell death (PCD) occurs in cattle during acute infection. Lymph nodes which are in the upper respiratory tract, tonsil, lung, turbinate, or trigeminal ganglia contain infected cells which undergo PCD. We hypothesize that PCD protects the host from viral spread and/or contributes to clinical symptoms associated with infection. An important viral regulatory gene, bICP0, is toxic and likely induces PCD. With respect to bICP0, the goals of this grant are to prove it induces PCD and map sequences which induce PCD and regulate viral gene expression. With respect to PCD in infected cattle, the goals of this proposal are to determine: 1) which cells undergo PCD, 2) whether reactivation occurs in neurons undergoing PCD, and 3) whether lymphoid cells are persistently infected with BHV-1. In summary, these studies will shed light on the role that PCD plays in pathogenesis and immunosuppression after infection.

9802070 Mechanisms of Strain Diversity in Transmissible Mink Encephalopathy

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Strengthening Award; Grant 98-35204-6409; $250,000; 3 Years

The transmissible spongiform encephalopathies (TSEs), or prion diseases, are fatal neurodegenerative diseases of animals and humans. These diseases include scrapie in sheep and goats, bovine spongiform encephalopathy (also known as "mad cow disease" or BSE) and transmissible mink encephalopathy as well as Creutzfeldt-Jakob disease in humans. For each of the TSE diseases there exists a variety of TSE agents, or strains, that cause slightly different clinical and pathological features. The aims of this research project are 1) to develop better methods for the identification and classification of TSE strains, 2) to understand the basis of TSE strain diversity, and 3) to assay drugs for the ability to block TSE agent formation. To accomplish these goals, we will examine strains of transmissible mink encephalopathy using *in vitro* models. These studies are important in order to safeguard U.S. livestock, food supplies, and biological products from infection or contamination with highly pathogenic or emerging TSE strains such as BSE. It is the goal of this study to understand how TSE strains differ in order to be able to rapidly identify and track TSE strains in order to effectively prevent their spread and minimize risk to animal and human health.

9802626 Role of Periplasmic PPlases in the Intracellular Survival of Salmonella

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Grant 98-35204-3442; $80,000; 1 Year

Salmonellosis is a major bacterial disease of poultry, pigs and cattle, and creates multi-million dollar losses in industries engaged in their production and care. In addition, human salmonellosis results in widespread suffering and substantial health care costs. Pathogenic *Salmonella* species initiate a productive infection by penetrating and growing within cells of the host's intestinal tract. Among several virulence factors, FkpA is one protein that helps *S. typhimurium* survive and replicate within epithelial cells and macrophages. This protein is a peptidyl-prolyl *cis-trans* isomerase (PPlase), and at least ten PPlases exist in *E. coli* and *S. typhimurium*. FkpA and three other PPlases are located in the bacterial periplasm and are therefore perfectly positioned to modify proteins that interact with the host during infection. The general goal of this research is to determine how FkpA and other periplasmic PPlases contribute to the establishment of intracellular infection by *S. typhimurium.* The specific objectives of this research are to: 1] clone, sequence and analyze the three remaining periplasmic PPlase genes from virulent *S. typhimurium;* 2] create chromosomal mutations that inactivate these genes; 3] determine the effects of these mutations on intracellular survival; and 4] screen for other proteins that may interact with these PPlases during infection. Clarifying this bacterium-versus-host relationship should contribute to the design of better treatments for *Salmonella* induced disease and to the development of more rational infection control measures.

9802282 Marek's Disease Virus Genes Associated with Cell-Mediated Immunity

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Grant 98-35204-6425; $235,000; 3 Years

Marek's disease (MD) is an economically important disease of chickens caused by Marek's disease herpesvirus (MDV). MD is characterized by the development of immunosuppression and lymphomas. Currently all commercial chickens in the USA are vaccinated against MD at 18 days of embryonation or at one day of age. However, the incidence of the disease is increasing probably as a result of the development of more pathogenic strains. Although vaccines have been used to protect against MD since 1970, the mechanism(s) for protection have not yet been elucidated. We have presented evidence that cell-mediated immune responses mediated by cytotoxic T cells (CTL) form an important part of the recovery from infection with MDV and the establishment of protective immunity. We have also identified some of the viral proteins that are recognized by the CTL. The purpose of this grant is to a) define the specific parts of the viral proteins or epitopes that are expressed on the surface of cells infected with MDV and therefore recognized by epitope-specific CTL, to develop *in vitro* approaches to cultivate epitope-specific CTL for use in passive transfer experiments, and c) to learn which viral proteins are important for CTL responses that are linked to the genetic resistance of specific chicken strains. We plan to use this information to develop recombinant vaccines expressing several epitopes important for protective immunity. These recombinant vaccines will be tested to learn if these vaccines induce enhanced protection compared to the current vaccines.

9802405 Effect of Clinical Mastitis on Milk Yield, Culling, and Profitability in Dairy Cows

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Grant 98-35204-6489; $240,000; 3 Years

Diseases, particularly mastitis, limit dairy industry productivity, not only because of the illness itself; but because currently there is no firm-specific method for deciding what to do with mastitic cows. Farmers currently lack a decision-making tool to use on their own individual farm to determine the economically optimal action. The dairy industry would be greatly improved by developing a systematic way to treat mastitis that can respond and adapt its problem-solving methodology to each individual farm situation. This is what we aim to do in our project. We will use state of the art statistical techniques to estimate the effect of clinical mastitis on milk production and on the length of time that dairy cows remain in the herd. Using these results, we will develop a decision tool that determines whether it is more profitable for a cow with clinical mastitis caused by a specific etiologic agent to be treated, not treated and kept, or culled. Data for this study will come from 5 (400-800 cows per herd), well managed New York State dairy herds. These herds will have information

on bacterial cultures for cases of clinical mastitis, as well as daily milk yields, other disorders, and costs for all cows. The knowledge gained from this study will help dairy farmers to more accurately estimate the impact of clinical mastitis on their herd's profitability and provide a more objective basis for making decisions regarding treatment and culling of their clinically mastitic cows.

9802528 Antigenic Conservation of *Anaplasma marginale* in Cell Culture

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Grant 98-35204-3528; $185,000; 2 Years

Anaplasma marginale, a rickettsial parasite of cattle, invades bovine erythrocytes and causes mild to severe anemia resulting in considerable economic loss worldwide. The erythrocytic stage of *A. marginale* has been the only source of *A. marginale* antigen available for use in serodiagnostic tests or vaccines. The erythrocyte derived antigen is often contaminated with bovine cell stroma and not cross-protective with isolates of *A. marginale* from different geographic areas. We recently propagated a Virginia isolate of *A. marginale* (VAMCC) in a continuous tick cell line. The VAMCC isolate identity, as determined by the molecular weight of the major surface protein la which varies among geographic isolates, was retained in culture. In this research, we will determine whether the antigenic composition of the VAMCC is conserved throughout the development cycle in culture and throughout transmission of *A. marginale* by ticks to cattle. We will determine whether the antigenic composition remains the same after successive passage in culture. We will propagate an Oklahoma isolate of *A. marginale* in culture and determine its antigenic composition. An understanding of the antigenic conservation of *A. marginale* derived from continuous cell culture is needed before cell culture-derived *A. marginale* is used an immunogen for a new vaccine or in future research. If the Oklahoma isolate retains the major surface proteins and its individual antigenic identity, as occurred with the Virginia isolate in culture, the cell culture-derived *A. marginale* may be useful as antigen for development of region specific vaccines.

9802107 Interactions of EPEC and EHEC with Host Cells

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Grant 98-35204-3426; $188,000; 2 Years

There are a number of bacteria that attach to the mucosal linings of farm animals, causing diarrhea and contamination of meat and any other food products that contact the feces or the contaminated meat. Ingestion of contaminated foods transfers the bacteria to human intestines. Six different groups of *Escherichia coli* can infect the gastrointestinal tract. Two of these: Enteropathogenic *E. coli* (EPEC) and Enterohemorrhagic *E. coli* (EHEC), attach to the surfaces of intestinal cells and induce the loss of the cells' microvilli, or finger-like projections of membrane, leading to diarrhea. EHEC also produces toxins which can lead to the death of humans who consume food contaminated with only a small number of these bacteria. Inside the intestinal cells, underneath the sites where EPEC and EHEC attach, are concentrations of actin, an important protein for many types of cellular motility. The membrane-attached bacteria, together with the actin and other intestinal cell proteins that bind actin, move along the surfaces of the infected cells. The broad objectives of this proposal are to understand how the infectious bacteria EPEC and EHEC rearrange host cell proteins to locomote on the surfaces of infected cells and exert their pathogenic effect. We will use a multidisciplinary approach to dissect the steps required to initiate and sustain motility of these bacteria on the surfaces of the infected cells. The proposed experiments will increase our basic knowledge of how these infectious bacteria cause diseases in animals and humans.

9802623 Vaccine Development for Enteric Diseases of Swine

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Grant 98-35204-6706; $150,000; 2 Years

Diarrhea is a major cause of mortality and morbidity in farm animals in the U.S. Enteric viruses like transmissible gastroenteritis virus (TGEV) can cause devastating infections in neonatal piglets resulting in near 100% mortality. There is a definite need for more effective vaccines against this and other enteric pathogens. The long term goal of this proposal is to develop a new polyvalent vaccine for swine by using the enteroadhesive 987P fimbriae of enterotoxigenic *Escherichia coli* as an immunogenic carrier system for inducing protection against various microbial agents of diarrhea. Fimbriae are highly immunogenic proteins, whose polymeric repetitive composition improves the immunogenicity of accompanying foreign antigens. The aim of this project focuses on genetically engineering the 987P fimbriae to carry immunogenic domains of TGEV. Animals will be immunized parenterally or orally with fimbriae displaying multiple domains of TGEV, or with *E. coli* or *Salmonella typhimurium* vaccine strains expressing these fimbriae. The immune response will be studied by measuring antibody production. More specifically, serum and mucosal antibodies in the intestines will be analyzed for virus neutralizing properties. In addition to serve as a model for better understanding the intestinal immune response to antigens presented by an enteroadhesive system like the 987P fimbriae or by an enteroinvasive system, like a Salmonella vaccine strain, the development of a 987P carrier system raises new prospects for safer and better vaccines protecting against a combination of major enteric pathogens simultaneously.

9802053 Role of Cytokines in Immune Response to Paratuberculosis Vaccination in Cattle

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Grant 98-35204-6705; $300,000; 3 Years

Paratuberculosis (Johne's disease) is a progressive and ultimately fatal intestinal infection in cattle that threatens the U.S. livestock industries. There is no practical treatment available, thus eradication of this disease depends on preventing the spread of infection. Unfortunately, currently available vaccines are not effective to prevent infection. This project will investigate the response of the bovine immune system to the bacterium that causes the disease, *Mycobacterium paratuberculosis*, and in particular, the reasons that available paratuberculosis vaccines fail to induce protective immunity in cattle. Newborn calves will be experimentally infected with paratuberculosis organisms simulating the natural infection that occurs on farms. The response of the calves' lymphocytes (white blood cells responsible for immunity) will be measured. Lymphocytes from the systemic blood circulation, and from lymph nodes draining the intestines will be tested. In particular, important proteins produced by the lymphocytes, known as cytokines, will be measured, and compared to those normally associated with resistance to other mycobacterial infections. Calves will be vaccinated, and the response to experimental infection in vaccinated calves will be measured, and cytokine production compared to that of unvaccinated calves. We then propose to manipulate the response to vaccination, by treating vaccinated calves with a recombinant cytokine, IL-12, to induce the calves' lymphocytes to develop the appropriate protective immune response for vaccination to be effective against paratuberculosis. The results of this study will lead to a more thorough understanding of the bovine immune response to paratuberculosis, and effective vaccine strategies for reducing the prevalence of this costly disease.

9802205 Rushmore Conference: Mechanisms in the Pathogenesis of Enteric Disease

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Grant 98-35204-6813; $5,000; 1 Year

This award supports the Second International Conference on the Mechanisms in the Pathogenesis of Enteric Diseases. Diarrheal diseases represent an important and difficult illness to prevent in animals and human beings. Diarrhea is a major health problem in pre-weaned pigs and cattle in the United States. Among cattle acute diarrhea affects approximately 10% of the 40 million beef and dairy calves born annually resulting in economic losses of approximately $100 million per year. Swine and cattle are also believed to be important sources of foodborne pathogens. Medical care and lost productivity due to foodborne illnesses costs the U.S. economy $2.9 to 6.7 billion annually. These pathogens reside in the intestines of animals and often contaminate meat products at slaughter. Many of the enteric diseases transmissible from animal to man invoke concern in urban and rural populations about the risks posed to water supplies by animal confinement units or feedlots. These concerns prevent expansion of livestock operations and result in lost economic opportunities for rural economies. The Rushmore Conference provides an environment for veterinary and medical scientists to exchange ideas and technologies that enhance our understanding of how bacteria, viruses and parasites cause enteric disease.

9802333 Development of RNA Ligands which Inhibit Visna Virus Replication

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Strengthening Award; Grant 98-35204-6636; $160,000; 2 Years

Lentiviruses are viruses that cause severe, often fatal diseases. The most well known lentivirus is the human "AIDS" virus, HIV. Many animal species (including horses, sheep, goats and cattle) can be infected by similar viruses. Visna virus is a lentivirus which causes fatal respiratory and nervous system diseases in sheep. In the l950s, a severe outbreak of visna disease occurred among sheep in Iceland; as a result, most of the sheep on the island had to be destroyed. Because there is currently no effective treatment or vaccine for any of the animal lentiviruses, they pose a major threat to animal health and cause significant economic loss world-wide. All lentiviruses contain a regulatory gene called *rev*. Because *rev* controls other viral genes, lentiviruses need a functional *rev* to replicate and cause disease. Our laboratory recently developed an RNA decoy molecule that inhibits visna *rev* funcdon and visna virus replication up to 85% in tissue culture. The immediate goal of this project is to use molecular biology techniques to alter the RNA decoy and make it a stronger inhibitor of visna virus replication. The long-term goal of this project is to express the altered RNA decoy in transgenic sheep, which should render these animals resistant to visna virus infection and disease. These studies are focused on production of disease-resistant livestock, which will directly benefit US agriculture. Because lentiviruses cause serious human diseases, these studies may also indirectly benefit human health.

9802610 Bovine-Specific Virulence Factors of *Salmonella typhimurium*

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Grant 98-35204-6735; $300,000; 3 Years

Salmonellosis is the most frequent food-borne illness in the US. In addition, Salmonella causes significant morbidity and mortality in cattle, resulting in financial loss to farmers. The recent emergence in the US of multi-resistant *S. typhimurium* strains has illustrated that the use of antibiotics will no longer combat salmonellosis effectively in the future. In order to devise alternatives for antibiotic

therapy for controlling or preventing Salmonella infections, an understanding of the fundamental factors that Salmonella uses to cause infection and disease is needed. While *S. typhimurium* virulence has been studied extensively in mice, little is known about genes allowing *S. typhimurium* to infect cattle. Calves infected with *S. typhimurium* develop severe diarrhea, with fatalities occurring from the resulting dehydration. In contrast, *S. typhimurium* does not cause diarrhea in mice but produces a systemic infection, designated murine typhoid, which is characterized by rapid bacterial growth in the reticuloendothelial system. The different signs of disease observed in mice and calves raise the question whether *S. typhimurium* uses different virulence mechanisms to produce illness in these hosts. To address this point we will systematically compare the sets of virulence genes necessary for *S. typhimurium* infection of cattle and mice. Bovine host range factors will be identified by investigating whether identical sets of virulence genes are required for infection of mice and calves. Bovine virulence factors will then be further characterized. The results from the proposed research will facilitate the development of improved strategies to prevent and treat infection.

9802265 The Molecular Basis for *Actinobacillus pleuropneumoniae* Capsule in Virulence

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Actinobacillus pleuropneumoniae (Ap) is the etiologic agent of swine pleuropneumonia. Ap isolates may be classified into 1 of 12 serotypes based on expression of a capsular polysaccharide, which is required for virulence. These 12 serotypes are known to vary in virulence and geographic predominance. Therefore, the type or amount of capsular polysaccharide produced by an Ap isolate may be the primary factor responsible for causing disease. Our long term goal is to determine the specific role of capsular polysaccharide in virulence, and the genetic basis for this control. We will achieve our goal by generating a mutant of Ap serotype 1 with a deletion in genes responsible for capsule biosynthesis. We will then clone and express in this mutant the capsular polysaccharide biosynthesis genes of Ap serotypes 1, 2, 3, 5, and 7, which are the serotypes that vary most in exotoxin production, virulence and geographic predominance. Expression of the serotype 1 capsule on a plasmid that makes a large number of copies will increase the amount of capsule produced compared to the parent strain. These experiments will therefore generate a set of strains that are identical except for the type or amount of capsule. We will then test the capability of these strains to cause pleuropneumonia in young, and evaluate the virulence of each strain using statistical methods. Understanding the genetic basis for how capsule controls virulence, and manipulation or inactivation of key capsule genes may result in improved vaccine strains for swine pleuropneumonia, as well as for other encapsulated pathogenic bacteria.

9802469 Roles of TNF-alpha and IL-12 in Macrophage Microbicidal Activity

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Agriculturally important animals are subject to infection by intracellular protozoan parasites, including *Babesia bovis, Theileria parve,* and *Neospora caninum*. Understanding the immune mechanisms by which parasitic infections are controlled and resolved is critical to the rational development of vaccines or treatment regimes. Host macrophages are active in the immune response through direct killing of parasites and through secretion of immunoregulatory cytokines, including tumor necrosis factor alpha (TNF-α) and interleukin 12 (IL-12). It may be possible to manipulate expression of these cytokines and subsequently improve the protective macrophage response; however, the specific roles of TNF-α and IL-12 must be determined in the bovine system. This research proposes the use of antisense oligonucleotides, oligonucleotides designed for complementarity with the AUG translation region of target mRNA, to specifically block translation of bovine TNF-α and IL-12. Specific antisense oligonucleotides cultured in vitro with bovine macrophages will abrogate secretion of these cytokines, and their absence will permit clear characterization of these cytokines. It is hypothesized that TNF-α and IL-12 are involved in bovine macrophage activation and parasite killing and that abrogation of expression will significantly impair activation and killing. On a large scale, this research is expected to yield a better understanding of TNF-α and IL-12-related regulation of bovine immune effector function against protozoan parasites. On a finer scale, the application of antisense oligonucleotides to abrogate the release of specific proteins is expected to be directly applicable in agricultural immunology and immunotherapy.

9802077 Snare Proteins That Mediate Secretion in Nematode Gut

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Parasitic nematodes cause numerous diseases that lead to morbidity and mortality of food production animals. In the absence of efficacious vaccines, chemotherapy is relied on both for treatment and control of these parasitic nematodes. Development of resistance against contemporary anthelmintics is well established in nematodes, and a continuing need exists to identify targets for control of these parasites. Evidence indicates that processes which regulate vesicle transport of gut membrane and secreted proteins have potential as targets to control parasitic nematode infections. Snare proteins (VRPs and SRPs) play a central role in regulating this process in other eucaryotic cells. Furthermore, tissue specific isoforms of these proteins exist, and there are reasons to believe that species specific VRP/SRP interactions might allow differential disruption of vesicle transport in the parasite versus the host. Here it is proposed to

investigate snare proteins that regulate this process in the gut of parasitic nematodes. However, the inability to genetically transfect parasitic nematodes presents an obstacle to elucidate molecular mechanisms of gut vesicle transport. Therefore, once specific proteins implicated in regulation of gut vesicle transport are identified for this parasite, related proteins will be obtained from the free-living nematode *Caenorhabditis elegans*. Transgenic *C. elegans* can be made, which provides a means to elucidate mechanisms of gut vesicle transport, once relevance to parasitic nematodes has been established. We have developed a gut specific approach with *H. contortus* that is unavailable for *C. elegans*. Therefore, the strengths of both the parasite and free-living nematode models should contribute synergistically to investigate basic processes that are likely to be shared by all parasitic nematodes.

9802091 Modulation of Bovine Immune Responses with Immunostimulatory DNA Sequences

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Devising strategies for effective vaccines and ways to safely modulate the immune response towards a desired effector pathway, is an important area of basic research, which will have practical applications for vaccine development in economically important domestic animals, including cattle. A new approach to immunizing cattle and other domestic animals is to use plasmid DNA vectors that encode a protective antigen, which then becomes expressed in the animal. In mice, such DNA vaccines are usually better than protein and adjuvant vaccines. One of the reasons for the success of DNA vaccines is the adjuvant property conferred by the DNA plasmid backbone itself. Specific sequences enhance the functions of antigen-presenting cells and antibody-producing cells, resulting in overall greater cell-mediated immunity, levels of immunoglobulin and production of the subclasses of antibodies important for protection against intracellular pathogens. This project will test the efficacy of such immunostimulatory DNA sequences in cattle. We have demonstrated that one sequence (AACGTT) stimulates bovine B-lymphocytes to proliferate, and that DNA obtained from *E. coli* and a protozoal parasite (*Babesia bovis*) induces B cell proliferation and IgG production. Our project will determine 1) the mitogenic effect of different CpG-containing oligonucleotides on B cell proliferation, IgG2 production, and expression of macrophage cytokines IL-12, IL-18, IFN-α and TNF-α, and 2) the capacity of a commercially available plasmid DNA vector and AACGTT sequences, present either in the vector DNA backbone or within an inserted commercially available plasmid DNA vector and *Babesia bovis* gene (*rap-1nt*), to stimulate macrophage cytokines that prime for enhanced cell-mediated immune responses.

9802480 Cloning and Characterization of the WC1 Counter-Receptor, BGAM

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Workshop cluster 1 (WC1) is a member of the scavenger receptor cysteine-rich (SRCR) superfamily (SRCRSF) of proteins that contain one or more of a highly conserved 110 AA motif. WC1 is expressed on an unique subset of γδ T cells that has undergone expansion in the course of evolution of ruminants and pigs. CD5 and CD6 are also members of the SRCRSF. CD5 is expressed on αβ T cells and WC1+ and WC1- γδ T cells. CD6 is only expressed on αβ T cells and WC1- γδ T cells. Limited information is available on the role of WC1, CD5, and CD6 in regulation and expression of immune responses. However, their composition suggests they serve as accessory molecules in modulating cell activation and function. The cytoplasmic tails of each protein contain motifs known to be involved in positive and negative signaling. Studies in our laboratory have focused on characterizing the WC1 molecule and determining its role in the function of WC1+ γδ T cells. WC1 is comprised of 11 SRCR domains, a hinge region, a transmembrane region, and a long cytoplasmic tail. The gene encoding WC1 is present in multiple copies. Analysis of expressed gene products by flow cytometry and a large panel of monoclonal antibodies has shown at least two isoforms can be expressed on mutually exclusive populations of cells. Studies to determine which SRCR domains serve as receptors for ligands (counter receptors; CR) for WC1 have shown that one receptor is present in domains 9-11. A fusion protein containing -these domains binds to a molecule expressed on macrophages and dendritic cells. We propose to clone and characterize the first identified CR for WC1 and determine its role in activation and function of WC1+ γδ T cells. Our current working hypothesis is: WC1 is an accessory molecule that is involved in cell signaling with cells that express the WC1 CR, bovine γδ T cell activation molecule (BGAM). The revised objectives for the project are: 1) Determine which SRCR domain contains the receptor for BGAM. 2) Clone the gene encoding BGAM, express the gene, and characterize BGAM. The third objective has been set aside until additional funds are obtained to pursue studies on the function of WC1 and BGAM.

9802272 Role of Type IV Pili in *Aeromonas salmonicida* Pathogenesis and use as a Vaccine

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Successful salmon aquaculture is important because while seafood demand is increasing, wild salmon are in decline due to environmental and habitat changes and over-fishing of dwindling resources. One important disease often encountered in salmon aquaculture is furunculosis, a debilitating and lethal infection caused by the bacterium *Aeromonas salmonicida*. Current vaccines have limited effectiveness, and epizootics are common in farmed fish in part due to high density rearing practices. We have cloned several genes required by *A. salmonicida* to express a specific class of pili, designated type IV, which are known virulence determinants in other

pathogenic bacteria and which have been used as vaccines against some animal infections. Pili (or fimbriae) are proteinaceous filaments that extend from many bacterial cells that mediate adherence and colonization of host cells by interactions with specific cell receptors. We propose to characterize the role of *A. salmonicida* type IV pili in colonization and virulence by first determining whether the pili are expressed during infections of salmon. The induction of specific anti-pili antibody will be measured in *A. salmonicida*-challenged fish to determine whether the fish immune system reacts to pili. We will then determine whether the pili play a role in pathogenesis by comparing the virulence of wild-type and pili-negative strains during experimental infections. Development of effective control measures requires a more complete understanding of pathogen and host factor interactions. If pili are important for *A. salmonicida* to infect fish, it is possible they could form the basis of an easily administered vaccine.

9802226 *Brucella abortus* Genes Activated Following Intracellular Invasion

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Macrophages serve as resident cells for *B. abortus*, but surprisingly little information is available detailing the bacterial mechanisms that permit intracellular invasion and persistence in infected cells. Understanding the mechanisms of *Brucella* persistence requires identifying the bacterial proteins that contribute to intracellular survival. Proteins induced in macrophages may undergo processing and presentation to T lymphocytes responsible for host resistance. Therefore, our long-term goal is to determine *B. abortus* genes induced or suppressed following infection and recognition of the encoded proteins by T lymphocytes. We have genetically engineered a screening system to detect *B. abortus* genes induced or suppressed following intracellular infection using a plasmid containing the promoterless modified green fluorescence gene (*gfp*). Insertion of a *B. abortus* DNA library in plasmids followed by *B. abortus* transformation resulted in many bacteria fluorescing green when exposed to uv light. Thus, we have constructed and tested the methodology to identify *B. abortus* promoters capable of activating the *gfp* gene. Now our Specific Objectives are: 1. To identify promoters induced or suppressed during infection, bacteria containing a plasmid will be plated on agar and screened for fluorescence. Individual fluorescent and non-fluorescent bacterial colonies will be added to macrophage monolayers, each well receiving a different bacterial colony followed by screening for loss or gain of fluorescence. *B. abortus* that switch promoter activity in macrophages will be reanalyzed on agar to assess promoter responsiveness to its environment. 2. To sequence the identified promoter and downstream gene, the *B. abortus* DNA fragment will be sequenced and homology with known sequences determined. Where open reading frames are not evident, the newly sequenced promoter will be used in Southern blot analysis of digested *B. abortus* DNA to identify open reading frames, as we have previously done. The specific objectives of this proposal are a first step in defining the mechanisms of *B. abortus* pathogenesis. The advantage of our system is the direct intracellular examination of bacterial promoters functionally associated with invasion and persistence. Using GFP as a reporter affords close and rapid examination of bacterial genes associated with infection that has not been accomplished previously with *Brucellae* and is essential in defining the dialog between *B. abortus* and the immune system following bacterial invasion.

9802466 Priming Mechanisms in Porcine Lung Disease

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An enhanced response, or priming, occurs as a result of prior exposure to infectious agents or their toxins, resulting in an increased pathophysiological response to subsequent infection. Bacteria or bacterial products, such as lipopolysaccharide (LPS) cause pulmonary inflammatory responses associated with release of chemicals such as, platelet-activating factor (PAF), eicosanoids, toxic oxygen metabolites, and increased interaction of white blood cells and endothelial cells through upregulation of adhesion molecule interactions between these cells. Previously, we have demonstrated that PAF can prime pigs for an increased pulmonary response to LPS, and PAF primes porcine white blood cells for increased release of superoxide anion, a toxic oxygen metabolite. Our proposal will test the hypothesis that exposure to inhaled LPS (a common route of LPS exposure in confinement housed pigs) will prime pigs for an increased negative cardiopulmonary response to subsequent intravenous LPS. Additionally, we will investigate the role of PAF as a mediator of LPS priming in this model. We further hypothesize that PAF and LPS priming upregulates white blood cell and endothelial cell adhesion molecules, thus increasing pathologic interactions of these cells within the lungs. Pigs will be treated with inhaled saline or LPS, followed by intravenous saline or LPS. The cardiopulmonary response, neutrophil superoxide release, and adhesion molecule expression will be evaluated, and PAF and eicosanoid concentrations in plasma will be measured. The focus of this proposal on interactions of inhaled LPS (a common route of LPS uptake in housed pigs) with inflammatory chemicals and adhesion molecules will further our understanding of basic disease processes in pigs, as well as impact on development of air quality in swine confinement facilities. This should help lessen the effects of respiratory disease on swine production.

9802394 Vesicular Stomatitis Virus Persistence in Convalescent Animals

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Vesicular stomatitis virus - New Jersey (VSV-NJ) infects cattle, horses, and swine causing severe disease and economic loss, infects humans causing an unpleasant but non-lethal disease, and infects wildlife with unmeasured consequences. Disease produced by VSV-NJ in cattle and swine can not be distinguished clinically from foot-and-mouth disease and thus demands substantial government resources. Since vaccines for VSV-NJ are lacking, control is by quarantine. The virus appears to have one or more insect or animal reservoirs in Central and South America and spreads on insects into temperate latitudes during the summer. Although insects are clearly involved in long-distance spread, the source of virus for insects remains unclear. And although infected animals can spread virus in the absence of insects, the duration of their infectivity is unclear. Lifelong extreme fluctuation in antibody levels suggest the virus persists in animals indefinitely. Previous research has shown clearly that the viral genome persists in animals for life. The goal of this research will be to carefully characterize the persistent VSV-NJ genome. Specifically, this research will determine which cells harbor persistent VSV-NJ RNA, define the length of this RNA, determine if viral proteins are associated with persistent RNA as might be expected if they are in a complex that can be replicated and expressed, determine if the persistent viral RNA is translated into proteins, and determine if persistent VSV-NJ RNA can reactivate into infectious virus. The results of this research should improve VSV-NJ control programs.

9802326 Immune Responses to Influenza Virus Infection and DNA Vaccination in Pigs

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Infection of pigs with influenza virus is an important problem for the swine industry, with high morbidity and economic losses during explosive outbreaks. In addition, pigs can serve as sources of influenza viruses for humans. Unfortunately, currently-available vaccines against influenza virus in pigs do not provide complete protection from infection. The goals of this research are to more fully understand the pig's immune responses to infection with influenza virus and to develop DNA-based approaches to vaccination. DNA-based vaccination refers to administration of the gene encoding an antigenically important protein, rather than the conventional approach of administering the protein itself. For our work, animals are vaccinated with the gene gun, a device that administers DNA into the skin on the surface of gold beads. Following infection or DNA vaccination, we will define antibody and cellular immune responses in pigs, including at the mucosal surfaces in the respiratory tract. In particular, we will employ a novel approach to enhance the pig's immune responses to DNA vaccination, namely coadministration of the gene encoding interleukin-6. Interleukin-6 is a chemical mediator secreted by cells that helps to regulate both antibody and cellular immune responses in the body. We have previously shown that co-administration of the gene for interleukin-6 and the hemagglutinin protein of influenza virus induces strong protection from influenza virus infection in mice. We will now extend this work to pigs, and additionally investigate the optimal timing for administration of DNA vaccine boosters and the effect of sequential DNA and traditional protein vaccination.